Exhibit 1

Qualifications

- Residency in Internal Medicine, University of Washington, 1992
- M.D., New York Medical College, 1989
- Ph.D. in Epidemiology, University of Washington, 1982
- M.A. in Medical Sociology, SUNY-Buffalo, 1976
- B.A., Boston University, 1974

Academic Appointments and Research

Fred Hutchinson Cancer Research Center

- Studies ways to prevent new and recurrent cancer
- Conducts epidemiologic research, identifying risk factors for cancers in women, such as breast and ovarian
- Studies prevention methods to reduce inflammation and other markers of cancer risk

University of Washington, Schools of Medicine and Public Health

- Research Professor, Epidemiology
- Adjunct Research Professor, Medicine - Gerontology and Geriatric Medicine
- Teaching and mentoring epidemiology students

Women's Health Initiative (WHI)

- Project Director for the Women's Health Initiative
- Oversaw the development of protocols, procedures, recruitment, interventions, follow-up, and outcomes, including ovarian cancer.

Published Research

- Published over 400 manuscripts in peer-reviewed medical and scientific journals
- 17 case-control studies, 127 cohort studies, 165 randomized clinical trials, 10 pooled analyses, 5 methods, multiple meta-analyses, and multiple reviews.

Advisor/Consultant for International Research Organizations

World Health Organization (WHO), International Agency for Research on Cancer (IARC)

- Contributor to 2002 IARC Handbook of Cancer Prevention: Physical Activity and Weight Control
- Working Group Member, completed systematic review
- Chaired section to identify biologically plausible mechanisms linking physical activity and weight control to cancer

World Cancer Research Fund/American Institute for Cancer Research

- Expert Panel Member of Continuous Update Project (CUP)
- CUP conducts research on cancer prevention and survivorship focused only on diet, nutrition, physical activity, and obesity

Advisor for Governmental Entities

U.S. Department of Health and Human Services

- Advisory Committees, 2008 and 2018 Physical Activity Guidelines
- Chair of the Cancer Subcommittee

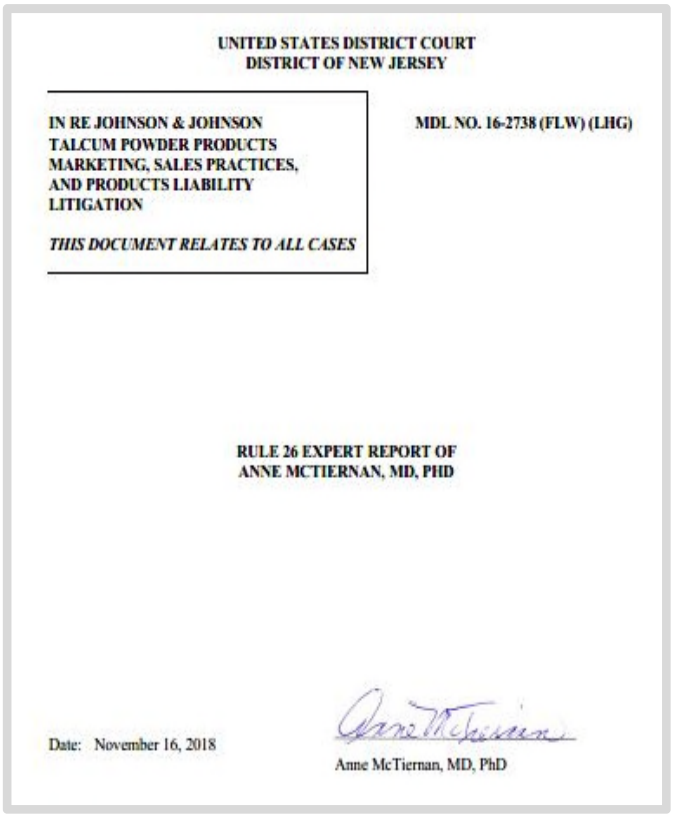
National Institutes of Health (NIH), National Cancer Institute (NCI)

- Principal Investigator, Seattle Transdisciplinary Research on Energetics and Cancer
- Program Reviewer for NCI research
- Reviewed grant applications

U.S. Department of Defense, U.S. Army Medical Research and Materiel Command

- Chair, Review of Congressionally Directed Medical Research Program Applications (CDMRP) Breast Cancer Research Program

Mandate and Opinion



UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

IN RE JOHNSON & JOHNSON TALCUM POWDER PRODUCTS MARKETING, SALES PRACTICES, AND PRODUCTS LIABILITY LITIGATION

MDL NO. 16-2738 (FLW) (LHG)

THIS DOCUMENT RELATES TO ALL CASES

RULE 26 EXPERT REPORT OF
ANNE MCTIERNAN, MD, PHD

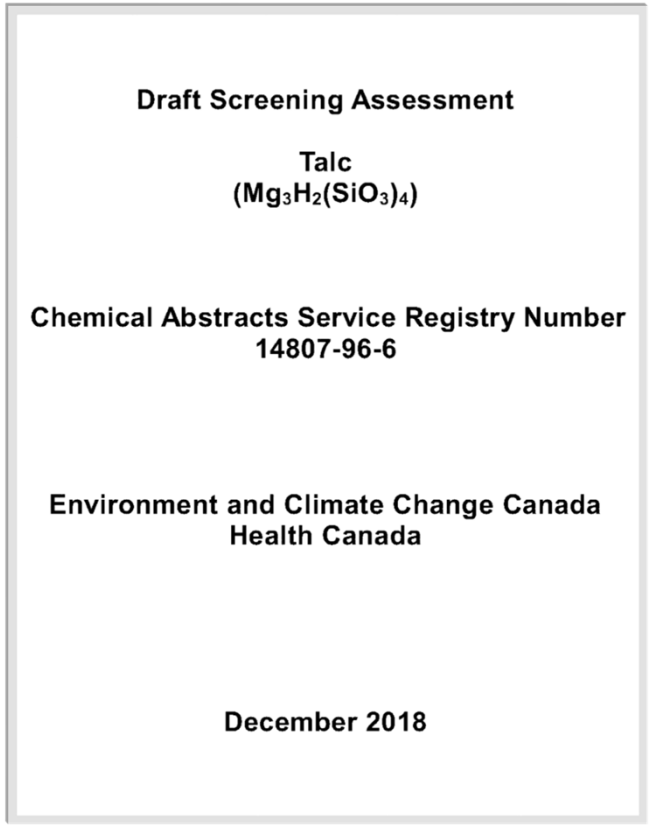
Date: November 16, 2018

Anne McTiernan, MD, PhD

Mandate: "[R]etained to review the current state of the scientific literature regarding talcum powder products and opine on whether those products cause ovarian cancer." (p. 3)

Opinions Reached: "In my opinion, as an epidemiologist and physician, stated to a reasonable degree of medical and scientific certainty, use of talcum powder products, including Johnson & Johnson Baby Powder and Shower to Shower, in the genital /perineal area can cause ovarian cancer. I base this opinion on the statistically significant elevated risk estimates (relative risk, odds ratios) seen when the epidemiologic data are combined, the pathological evidence, the consistency of results across geographic areas and in different race/ethnic groups, the evidence of a positive dose-response effect, and the plausible biological mechanism." (p. 9)

Opinions Shared: Health Canada



Draft Screening Assessment

Talc
($Mg_3H_2(SiO_3)_4$)

Chemical Abstracts Service Registry Number
14807-96-6

Environment and Climate Change Canada
Health Canada

December 2018

Expert Comments (Submitted February 2, 2019)

- The Health Canada assessment states "[t]he meta-analysis of the available human studies in the peer reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect …"
- "This conclusion agrees with my own: …. There have been 33 scientific publications from epidemiologic studies, as well as a pooled analysis and several meta-analyses that combined data across multiple studies. The meta-analyses consistently showed that women who have ever used talcum powder products in the genital/perineal area have a statistically significant 22-31% increased risk of developing epithelial ovarian cancer overall compared with women who reported never using these products. These comprehensive combined analysis also show strong evidence of increased risk of ovarian cancer with increasing number of estimated lifetime applications of talcum powder products in the perineal/genital area."

Health Canada Response to Submitted Comments

On Feb 21, 2019, at 5:53 AM, Hancock, Scott (HC/SC) [redacted] wrote:

Hello Dr. McTiernan. I am a senior manager involved in the development of the Talc Screening Assessment here at Health Canada. I would like to thank you for your submission related to our DSAR. In the coming months, my team will be reviewing the more than 100 public comments we received as part of our consultation process. To that end, we typically meet with concerned stakeholders who have requested meetings. In this case, there are several industry groups who have asked for meetings to discuss their comments on the Draft Screening Assessment for Talc. I was wondering, if you would be available for phone calls, email exchanges, or even short term contracts to help offer scientific opinion on some arguments we have received as we work towards rewriting our screening assessment? Your expertise in this area would be most welcome

Thanks in advance

Scott Hancock
Acting Senior Manager,
ESRAB, SED, Health Canada
[redacted]

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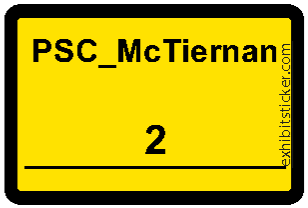


Opinions Shared: Congressional Testimony

<u>March 12, 2019: Hearing on Health Risks of Carcinogens in Consumer Products</u>
House Committee on Oversight and Reform
Subcommittee on Economic and Consumer Policy

- My review identified 38 high-quality epidemiologic studies conducted over the past 40 years. These studies asked women about their use of talcum powder products in the genital area, and tested associations with risk of ovarian cancer. Together, these studies included over 14,000 women with epithelial ovarian cancer (the most common type) and an even greater number of women without ovarian cancer.
- Summarizing data from all of the published studies consistently shows that women who had ever used talcum powder products in the genital area had a statistically significant 22-31% increased risk of developing epithelial ovarian cancer compared with women who had never used them.
- These combined analyses showed that increasing amount of exposure to talcum powder products in the genital area resulted in increasing risk of developing epithelial ovarian cancer.

Congressional Testimony

- Published laboratory and clinical studies provide evidence that in humans, talc can migrate from the genital area to the ovaries and fallopian tubes. Talc has been shown to cause an inflammatory response in the human body. Elevated levels of inflammation in women are associated with increased risk of ovarian cancer. All of this provides a biologically plausible pathway by which talcum powder product exposure can cause ovarian cancer.
- Given the frequency with which asbestos has been found in cosmetic and personal use talc products, I reviewed the literature on the epidemiology of asbestos and risk of ovarian cancer. In 2012, the International Agency for Research on Cancer stated that a causal association between exposure to asbestos and cancer of the ovary was clearly established. That agency has also classified fibrous talc as a Class 1 carcinogen – the most dangerous level of carcinogen.

Methodology

Formulate Question

- What is the association between talcum powder products and ovarian cancer?
- Can use of talcum powder products cause ovarian cancer?

Systematic Review

- Defined search terms
- Pub Med Search
- Applied inclusion and exclusion criteria
- Identified 38 relevant original and peer-reviewed epidemiologic publications
- Updated Search

Data Review

- Reviewed relevant epidemiologic studies
- Considered statistical data, strengths/weaknesses of study type, effect of possible bias, chance, confounding, differences in exposure measures, etc.
- Considered dose response
- Considered data from non-epidemiologic lines of evidence, such as animal, cell, clinical, and pathological studies
- Considered non-talc components of talcum powder products and impact on carcinogenicity, such as asbestos, fibrous talc, heavy metals, and fragrances

Data Extraction

- Extracted data into four tables (case-control, cohorts, meta-analyses, and pooled)
- Extracted study characteristics, such as study year, nationality, population type, number of cases, number of non-cases, average age, follow-up years, dose response, relative risk, confidence intervals, and cancer subtype

Bradford Hill Analysis

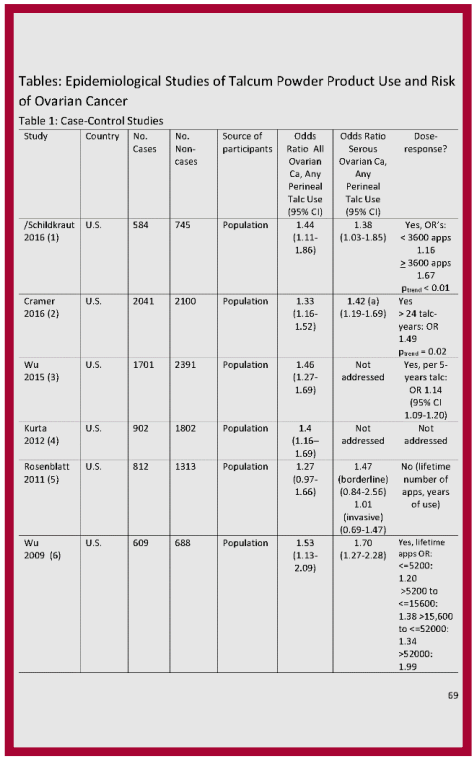
- Used independent judgment in assessing causality from the totality of evidence

Weighed Relevant Evidence - and - Reached Conclusion

Epidemiologic Study Designs Addressing Talcum Powder Products and Ovarian Cancer

- Case-Control
- Cohort
- Meta-Analysis
- Pooled

Expert Report - Data Extraction Tables (p. 69-77)



Tables: Epidemiological Studies of Talcum Powder Product Use and Risk of Ovarian Cancer

Table 1: Case-Control Studies

Study	Country	No. Cases	No. Non-cases	Source of participants	Odds Ratio All Ovarian Ca, Any Perineal Talc Use (95% CI)	Odds Ratio Serous Ovarian Ca, Any Perineal Talc Use (95% CI)	Dose-response?
/Schildkraut 2016 (1)	U.S.	584	745	Population	1.44 (1.11-1.86)	1.38 (1.03-1.85)	Yes, OR's: < 3600 apps 1.16 ≥ 3600 apps 1.67 $p_{trend} < 0.01$
Cramer 2016 (2)	U.S.	2041	2100	Population	1.33 (1.16-1.52)	1.42 (a) (1.19-1.69)	Yes > 24 talc-years: OR 1.49 $p_{trend} = 0.02$
Wu 2015 (3)	U.S.	1701	2391	Population	1.46 (1.27-1.69)	Not addressed	Yes, per 5-years talc: OR 1.14 (95% CI 1.09-1.20)
Kurta 2012 (4)	U.S.	902	1802	Population	1.4 (1.16–1.69)	Not addressed	Not addressed
Rosenblatt 2011 (5)	U.S.	812	1313	Population	1.27 (0.97-1.66)	1.47 (borderline) (0.84-2.56) 1.01 (invasive) (0.69-1.47)	No (lifetime number of apps, years of use)
Wu 2009 (6)	U.S.	609	688	Population	1.53 (1.13-2.09)	1.70 (1.27-2.28)	Yes, lifetime apps OR: <=5200: 1.20 >5200 to <=15600: 1.38 >15,600 to <=52000: 1.34 >52000: 1.99

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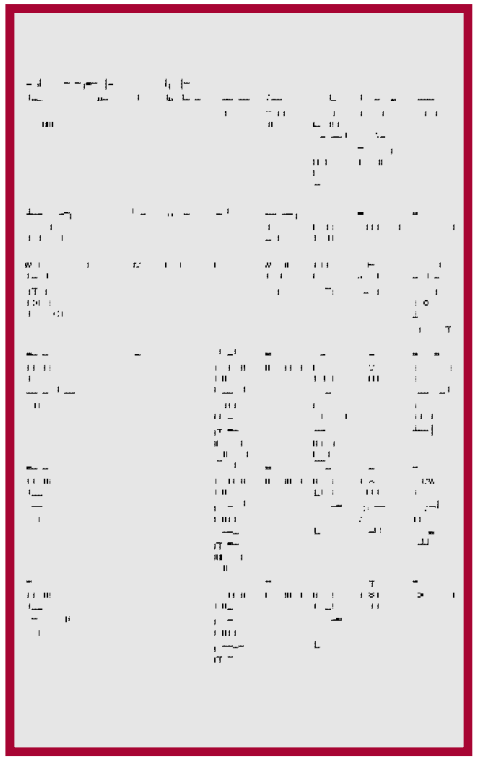
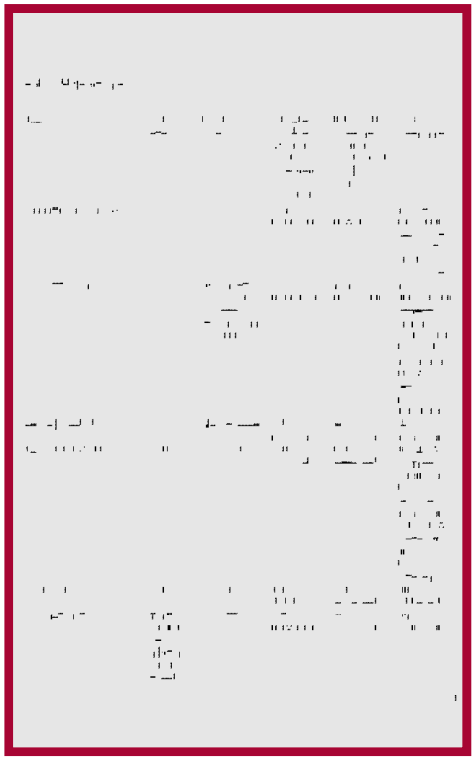
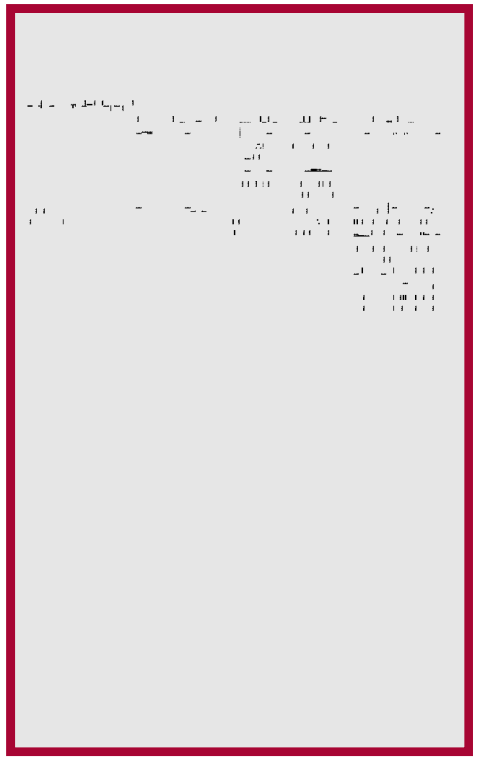
Case-Control (p. 69-72)



Cohort (p. 73-74)



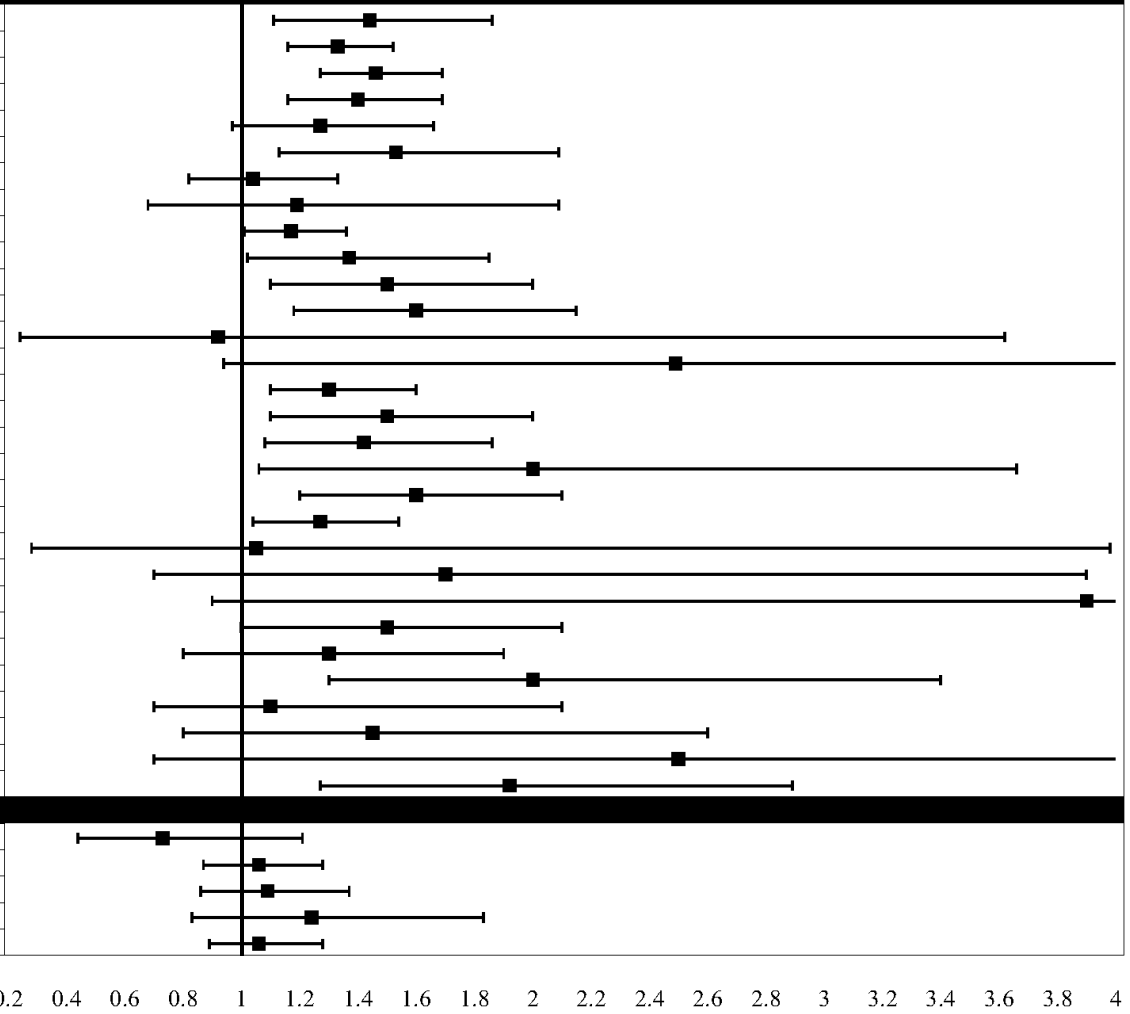
Meta-Analyses (p. 75-76)



Pooled (p. 77)

Case-Control and Cohort Studies (All Ovarian) (1982-2016)

Case-Control Study	Natl	Cases	Ctrls	Pop	DR	RR	CI L	CI U
Schildkraut 2016	US	584	745	Pop.	Yes	1.44	1.11	1.86
Cramer 2016	US	2041	2100	Pop.	Yes	1.33	1.16	1.52
Wu 2015	US	1791	2391	Pop.	Yes	1.46	1.27	1.69
Kurta 2012	US	902	1802	Pop.	N/A	1.4	1.16	1.69
Rosenblatt 2011	US	812	1313	Pop.	No	1.27	0.97	1.66
Wu 2009	US	609	688	Pop.	Yes	1.53	1.13	2.09
Moorman 2009 (white)	US	1114	1086	Pop.	N/A	1.04	0.82	1.33
Moorman 2009 (black)	US	1114	1086	Pop.	N/A	1.19	0.68	2.09
Merritt 2008	Aus	1576	1509	Pop.	Yes	1.17	1.01	1.36
Mills 2004	US	256	1122	Pop.	No	1.37	1.02	1.85
Ness 2000	US	767	1367	Pop.	Incpl	1.5	1.1	2.0
Cramer 1999	US	563	523	Pop.	Yes	1.6	1.18	2.15
Wong 1999	US	499	755	Hosp.	Incpl	0.92	0.24	3.62
Godard 1998	Can	170	170	Pop.	N/A	2.49	0.94	6.58
Green 1997	Aus	824	855	Pop.	Incpl	1.3	1.1	1.6
Cook 1997	US	313	422	Pop.	No	1.5	1.1	2.0[1]
Chang 1997	Can	450	564	Pop.	No	1.42	1.08	1.86
Shushan 1996	Isr	200	408	Pop.	N/A	2	1.06[2]	3.66[2]
Cramer 1995	US	450	454	Pop.	N/A	1.6	1.2	2.1
Purdie 1995	Aus	824	860	Pop.	N/A	1.27	1.04	1.54
Tzonou 1993	Gre	189	200	Hosp.	N/A	1.05	0.28	3.98
Rosenblatt 1992	US	77	46	Hosp.	Yes	1.7	0.7	3.9
Chen 1992	Chn	112	224	Pop.	N/A	3.9	0.9	10.63
Harlow 1992	US	235	239	Pop.	Yes	1.5	1.0	2.1
Booth 1989 (daily)	UK	235	451	Hosp.	Yes	1.3	0.8	1.9[1]
Booth 1989 (weekly)	UK	235	451	Hosp.	Yes	2.0	1.3	3.4
Harlow 1989	US	116	158	Pop.	N/A	1.1	0.7	2.1
Whittemore 1988	US	188	539	H&P	Yes	1.45	0.81[1]	2.60[1]
Hartge 1983	US	135	171	Hosp.	N/A	2.5	0.7	10.0
Cramer 1982	US	215	215	Pop.	N/A	1.92	1.27	2.89
Cohort Studies	**Natl**	**Cases**	**Non**	**Flw Up**	**DR**	**RR**	**CI L**	**CI U**
Gonzalez (2016)	US	154	41,500	6.6	N/A	0.73	0.44	1.2[1]
Houghton (2014)	US	429	61,147	12.4	Incpl	1.06	0.87	1.28
Gertig (2000)	US	307	78,323	N/A	Incpl	1.09	0.86	1.37
Gates (2008)	US	210	600	N/A	Yes	1.24	0.83	1.83
Gates (2010)	US	797	78,323?	N/A	N/A	1.06	0.89	1.28



[1] Corrected data-point from study text (report figure: Cook 1997 CI Upper 2.3; Gonzalez CI Upper 1.21; Booth 1989 CI Upper 1.0; Whittemore CI p=0.06).

[2] Corrected data-point from defense expert report(s) (report figure: p=0.04).

Case-Control and Cohort Studies (Serous)

Study	RR	CI L	CI U
Schildkraut 2016 (serous)	1.38	1.03	1.85
Cramer 2016 (serous)	1.42	1.19	1.69
Rosenblatt 2011 (borderline)	1.47	0.84	2.55[1]
Rosenblatt 2011 (invasive)	1.01	0.69	1.47
Wu 2009 (serous)	1.70	1.27	2.28
Merritt 2008 (serous)	1.21	1.03	1.44
Mills 2004 (serous)	1.77	1.12	2.81[1]
Cramer 1999 (borderline)	1.38	0.82	2.31
Cramer 1999 (invasive)	1.70	1.22	2.39
Wong 1999 (serous)	1.20	0.7	2.1
Cook 1997 (serous)	1.70	1.1	2.5
Chang 1997 (serous)	1.336[1]	0.96	1.85
Harlow 1992 (serous)	1.4	0.9	2.2
Cohort Studies	**RR**	**CI L**	**CI U**
Houghton (2014) (serous)	1.13	0.84	1.51
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Gates (2008) (serous)	1.48	0.82	2.68
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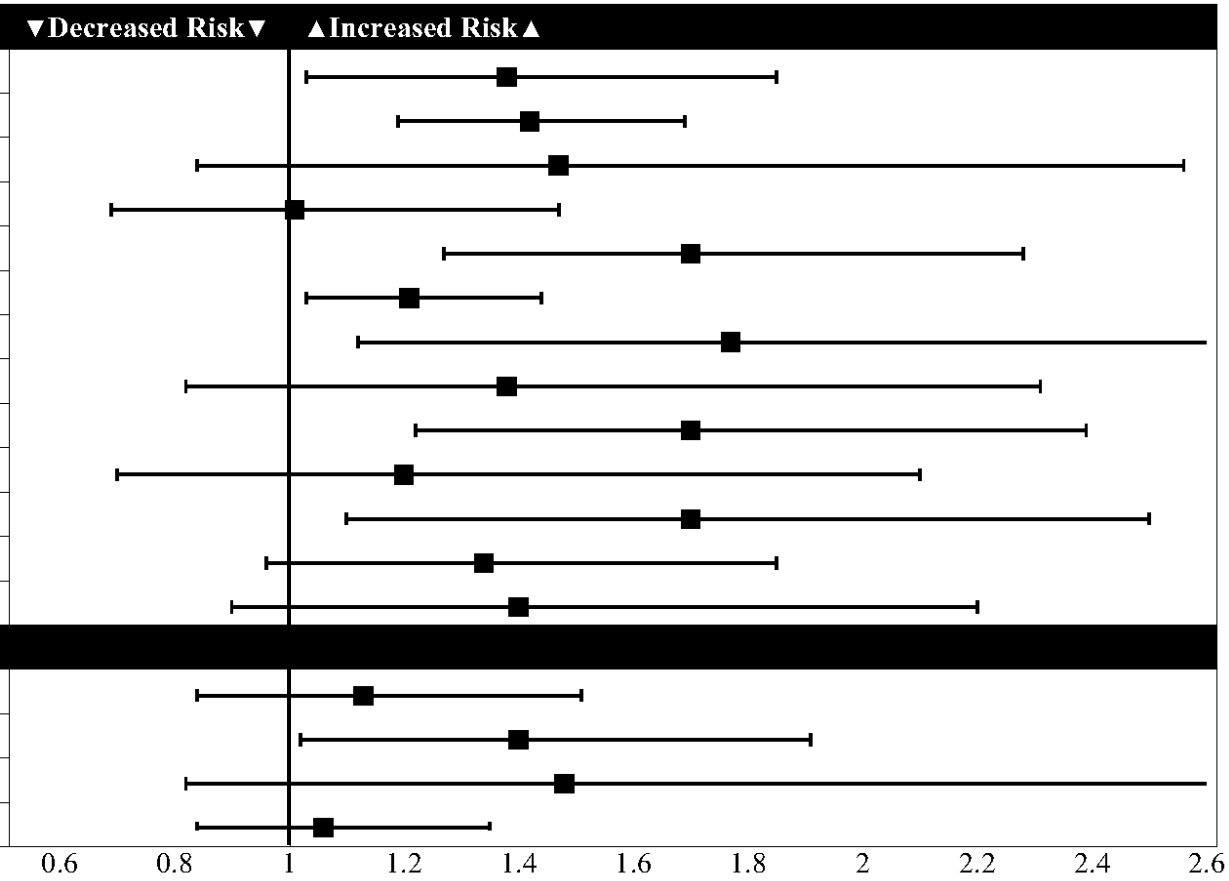

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Case-Control and Cohort Studies (All Ovarian)

Case-Control Study	Natl	Cases	Ctrls	Pop	DR	RR	CI L	CI U
Schildkraut 2016	US	584	745	Pop.	Yes	1.44	1.11	1.86
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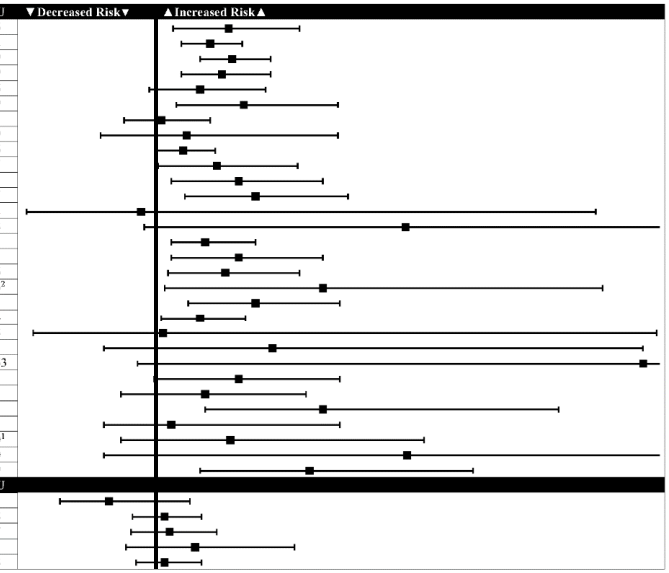

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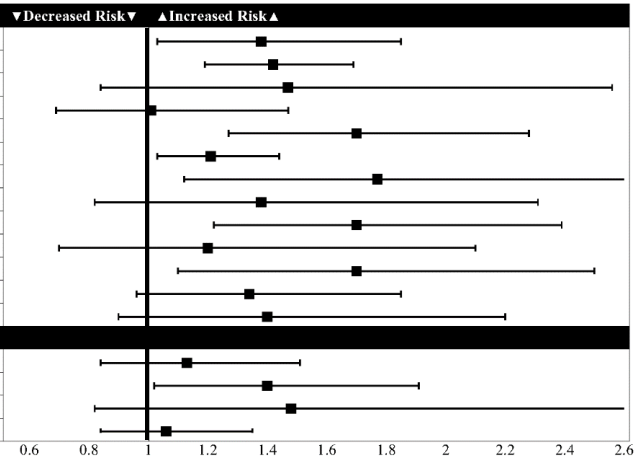
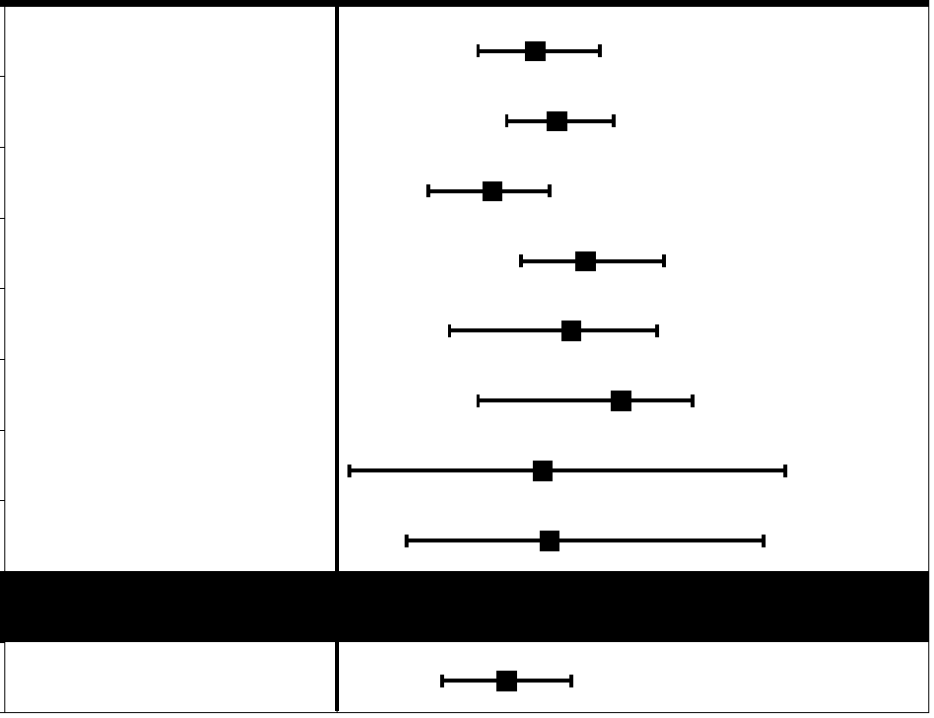


[1] Corrected data-point from study text (report figure: Rosenblatt 2011 CI Upper 2.56; Mills 2004 CI Upper 2.80; Chang 1997 RR 1.34).

Meta-Analyses and Pooled Studies (All Ovarian)

Study	#Studies	#Cases	DR	RR	CI L	CI U
Taher (2018)[1]	27	17,149	Yes	1.28	1.2	1.37
Penninkilampi (2018)	27	14,311	Yes	1.31	1.24	1.39
Berge (2018)[2]	27	N/A[3]	Yes	1.22	1.13	1.3
Langseth (2008)	20	N/A[3]	N/A	1.35	1.26	1.46
Huncharek (2003)	16	5260	No[4]	1.33	1.16	1.45
Cramer (1999)	14	3834	N/A	1.4	1.2	1.5
Gross (1995)	10[5]	1509	N/A	1.29	1.02	1.63
Harlow (1992)	6	1106	N/A	1.3	1.1	1.6
Pooled	**#Studies**	**#Cases**	**DR**	**RR**	**CI L**	**CI U**
Terry (2013)	8	8,525	Yes	1.24	1.15	1.33



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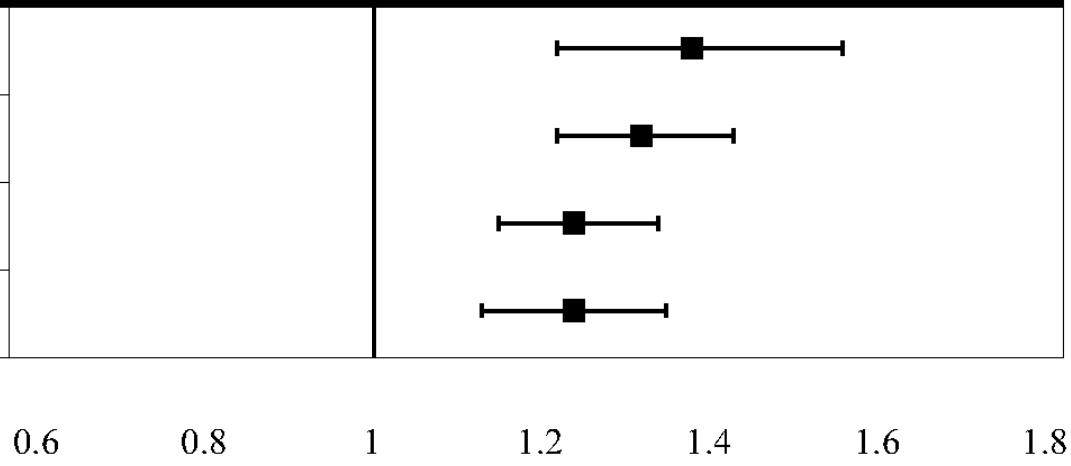
[5] N=5 studies with adjusted data and limited to epithelial ovarian cancers.

Meta-Analyses (Serous)

Study	RR	CI L	CI U
Taher (2018) (serous)[1]	1.38	1.22	1.56
Penninkilampi (2018) (serous)	1.32	1.22	1.43
Berge (2018) (serous)[2]	1.24	1.15	1.34
Terry (2013) (invasive)	1.24	1.13	1.35



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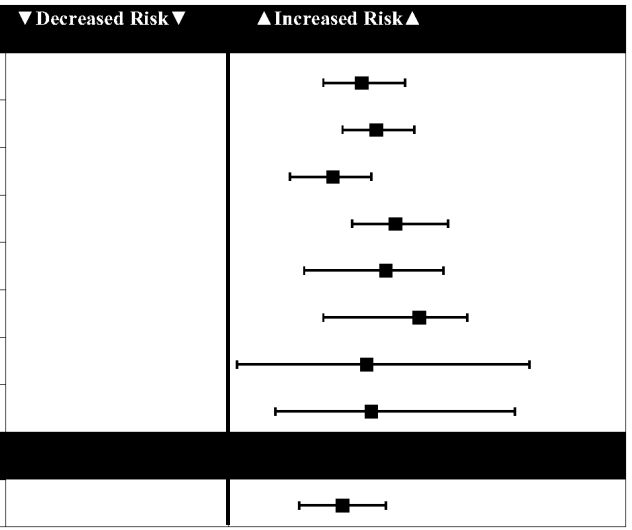
Meta-Analyses and Pooled Studies (All Ovarian)

Study	#Studies	#Cases	DR	RR	CI L	CI U
Taher (2018)[1]	27	17,149	Yes	1.28	1.2	1.37
Penninkilampi (2018)	27	14,311	Yes	1.31	1.24	1.39
Berge (2018)[2]	27	N/A[3]	Yes	1.22	1.13	1.3
Langseth (2008)	20	N/A[3]	N/A	1.35	1.26	1.46
Huncharek (2003)	16	5260	No[4]	1.33	1.16	1.45
Cramer (1999)	14	3834	N/A	1.4	1.2	1.5
Gross (1995)	10[5]	1509	N/A	1.29	1.02	1.63
Harlow (1992)	6	1106	N/A	1.3	1.1	1.6
Pooled	**#Studies**	**#Cases**	**DR**	**RR**	**CI L**	**CI U**
Terry (2013)	8	8,525	Yes	1.24	1.15	1.33



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Taher (2018) (serous)[1]	1.38	1.22	1.56
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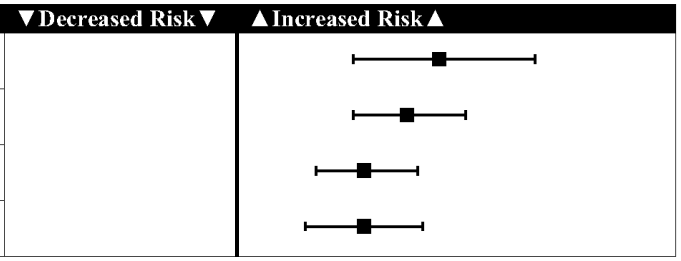

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Cohort: Nurses' Health Study (NHS)

Gertig (2000)

- **Overview:** Original cohort of 121,700 registered nurses (30-55 yrs old, study entry)
- **Participants:** 78,630 from larger cohort with 307 Ovarian Cancer cases
- **Data Collection Period:** 1982 through1996
- **Questionnaire:** Self-Administered
  - Talc question introduced in 1982, not included in original 1976 questionnaire
  - Question grouped all powders (including talc) in asking about "ever use"
  - Not updated
- **Dose Response Data:**
  - Frequency obtained, not duration data
- **Findings:**
  - All OC RR: 1.09 (CI: 0.86-1.37) (DR: No)
  - Serous OC RR: 1.40 (CI: 1.02-1.91) (DR: Yes)
- **Strengths:** Prospective nature limits recall bias
- **Weaknesses:**
  - Missing data
  - Incomplete dose-response data, no duration
  - Exposure misclassification (info not updated)
  - Insufficient power to find RR
  - Studied nurses only - not representative of population (limited generalizability)

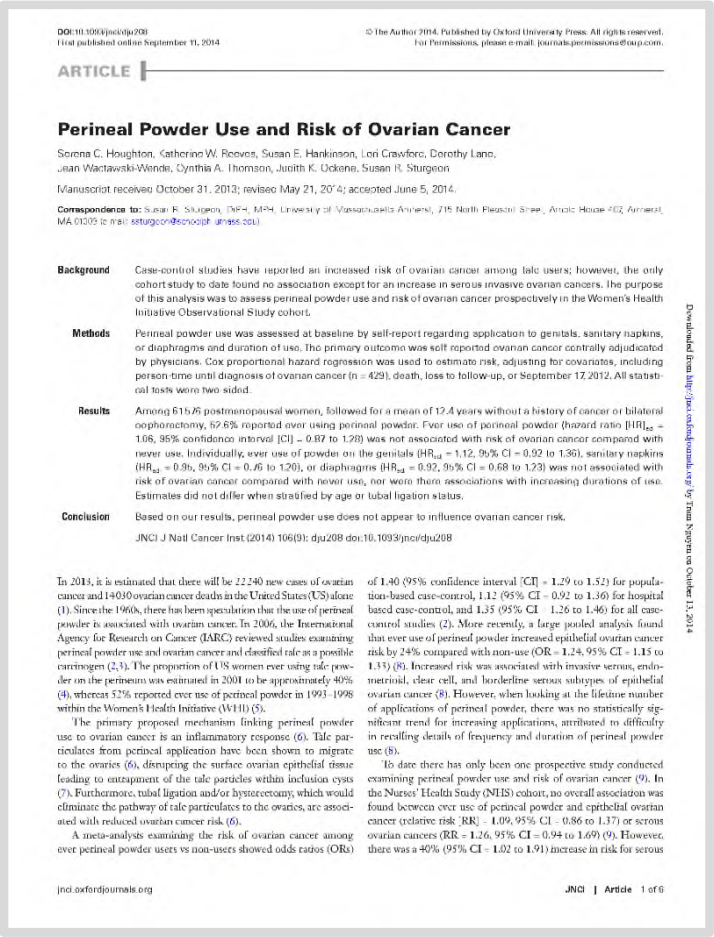
Gates 2008

- **Overview:** Nested case control study **Participants:** 210 Ovarian Cancer cases (unclear how chosen), 600 controls
- **Data Collection Period:** For NHS, see Gertig 2000
- **Questionnaire:** For NHS, see Gertig 2000
- **Dose Response Data:**
  - Less than 1 wk RR: 0.98
  - 1-6 wk RR: 1.01
  - Greater than 6 wk RR: 1.44
- **Findings:**
  - Combined "never use" with less than 1wk use
  - All OC (perineal talc use) greater than 1 wk v less than1 wk RR: 1.24 (0.83-1.83)
  - Serous invasive OC (perineal use) greater than 1 wk v less than 1wk RR: 1.48 (0.82-2.68)
- **Note:** Study focused on genetic analysis; not included in meta-analyses

Gates 2010

- **Overview:** Addresses multiple OC risk factors
- **Participants:** 797 Ovarian Cancer cases
- **Data Collection Period:** Occurrence of OC evaluated from 1983 through 2006
- **Questionnaire:**
  - Powder/Talc use asked in 1982
  - Not updated
- **Dose Response Data:**
  - Not addressed
  - Frequency obtained, no lifetime use
- **Findings:**
  - Combined never use w/less than 1wk users
  - Greater than1wk v less than 1wk use RR:1.06(0.89-1.28)
  - Greater than 1 wk v less than1wk Serous OC RR: 1.06 (0.84-1.35)
- **Strengths:** Prospective nature limits recall bias
- **Weaknesses:**
  - Changed classification of exposure, combining non-users with <1week users
  - Did not address dose response
  - Missing data
  - Exposure misclassification(info not updated)
  - Insufficient power to RR
  - Studied nurses only - limited generalizability

Cohort: Women's Health Initiative (WHI)



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ARTICLE

Perineal Powder Use and Risk of Ovarian Cancer

Serena C. Houghton, Katherine W. Reeves, Susan E. Hankinson, Lori Crawford, Dorothy Lane, Jean Wactawski-Wende, Cynthia A. Thomson, Judith K. Ockene, Susan R. Sturgeon

Manuscript received October 31, 2013; revised May 21, 2014; accepted June 5, 2014.

Correspondence to: Susan R. Sturgeon, DrPH, MPH, University of Massachusetts Amherst, 715 North Pleasant Street, Arnold House 402, Amherst, MA 01003 (e-mail: ssturgeon@schoolph.umass.edu).

Background Case-control studies have reported an increased risk of ovarian cancer among talc users; however, the only cohort study to date found no association except for an increase in serous invasive ovarian cancers. The purpose of this analysis was to assess perineal powder use and risk of ovarian cancer prospectively in the Women's Health Initiative Observational Study cohort.

Methods Perineal powder use was assessed at baseline by self-report regarding application to genitals, sanitary napkins, or diaphragms and duration of use. The primary outcome was self-reported ovarian cancer centrally adjudicated by physicians. Cox proportional hazard regression was used to estimate risk, adjusting for covariates, including person-time until diagnosis of ovarian cancer (n = 429), death, loss to follow-up, or September 17, 2012. All statistical tests were two-sided.

Results Among 61 576 postmenopausal women, followed for a mean of 12.4 years without a history of cancer or bilateral oophorectomy, 67.6% reported ever using perineal powder. Ever use of perineal powder (hazard ratio [HR]$_{ec}$ = 1.06, 95% confidence interval [CI] = 0.87 to 1.28) was not associated with risk of ovarian cancer compared with never use. Individually, ever use of powder on the genitals (HR$_{ec}$ = 1.12, 95% CI = 0.92 to 1.36), sanitary napkins (HR$_{ec}$ = 0.96, 95% CI = 0.76 to 1.20), or diaphragms (HR$_{ec}$ = 0.92, 95% CI = 0.68 to 1.23) was not associated with risk of ovarian cancer compared with never use, nor were there associations with increasing durations of use. Estimates did not differ when stratified by age or tubal ligation status.

Conclusion Based on our results, perineal powder use does not appear to influence ovarian cancer risk.

JNCI J Natl Cancer Inst (2014) 106(9): dju208 doi:10.1093/jnci/dju208

In 2013, it is estimated that there will be 22 240 new cases of ovarian cancer and 14 030 ovarian cancer deaths in the United States (US) alone (1). Since the 1960s, there has been speculation that the use of perineal powder is associated with ovarian cancer. In 2006, the International Agency for Research on Cancer (IARC) reviewed studies examining perineal powder use and ovarian cancer and classified talc as a possible carcinogen (2,3). The proportion of US women ever using talc powder on the perineum was estimated in 2001 to be approximately 40% (4), whereas 52% reported ever use of perineal powder in 1993–1998 within the Women's Health Initiative (WHI) (5).

The primary proposed mechanism linking perineal powder use to ovarian cancer is an inflammatory response (6). Talc particulates from perineal application have been shown to migrate to the ovaries (6), disrupting the surface ovarian epithelial tissue leading to entrapment of the talc particles within inclusion cysts (7). Furthermore, tubal ligation and/or hysterectomy, which would eliminate the pathway of talc particulates to the ovaries, are associated with reduced ovarian cancer risk (6).

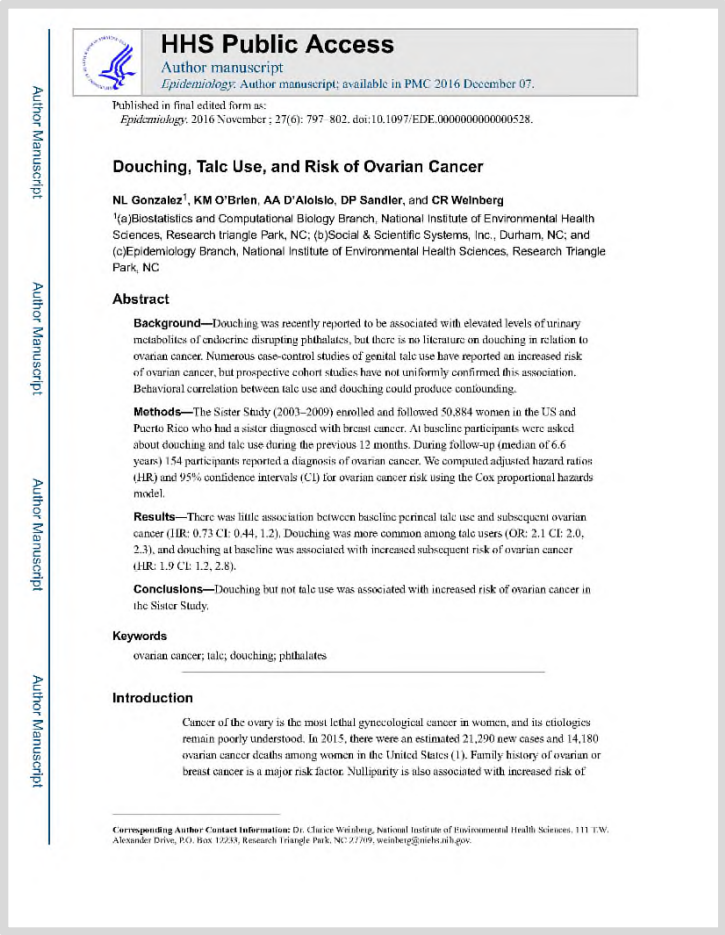
A meta-analysis examining the risk of ovarian cancer among ever perineal powder users vs non-users showed odds ratios (ORs) of 1.40 (95% confidence interval [CI] = 1.29 to 1.52) for population-based case-control, 1.12 (95% CI = 0.92 to 1.36) for hospital based case-control, and 1.35 (95% CI = 1.26 to 1.46) for all case-control studies (2). More recently, a large pooled analysis found that ever use of perineal powder increased epithelial ovarian cancer risk by 24% compared with non-use (OR = 1.24, 95% CI = 1.13 to 1.33) (8). Increased risk was associated with invasive serous, endometrioid, clear cell, and borderline serous subtypes of epithelial ovarian cancer (8). However, when looking at the lifetime number of applications of perineal powder, there was no statistically significant trend for increasing applications, attributed to difficulty in recalling details of frequency and duration of perineal powder use (8).

To date there has only been one prospective study conducted examining perineal powder use and risk of ovarian cancer (9). In the Nurses' Health Study (NHS) cohort, no overall association was found between ever use of perineal powder and epithelial ovarian cancer (relative risk [RR] = 1.09, 95% CI = 0.86 to 1.37) or serous ovarian cancers (RR = 1.26, 95% CI = 0.94 to 1.69) (9). However, there was a 40% (95% CI = 1.02 to 1.91) increase in risk for serous

Houghton (2014)

- **Overview:** 93,000 women at study entry, baseline enrollment age of 63.3 yrs (post-menopausal); Dr. Mc Tiernan, Project Director and Co-Investigator
  Participants: 429 Ovarian Cancer cases
- **Data Collection Period:** 1993 through 1998 (enrollment)
- **Questionnaire:** Self-Administered
  - Talc use data obtained at time of enrollment, but not updated
  - Follow-up 12.4 yrs
- **Dose Response:** Not observed
  - Asked about duration only, no frequency data
- **Findings:**
  - All OC (perineal use) HR: 1.12 (0.92-1.36)
  - Serous OC (any powder use) HR: 1.13 (0.84-1.51)
- **Strengths:** Prospective nature limits recall bias
- **Weaknesses:**
  - Missing Data
  - Exposure not updated
  - Insufficient power to detect RR
  - Incomplete does response analysis (did not ask about frequency)
  - Limited generalizability

Cohort: Sister Study



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Douching, Talc Use, and Risk of Ovarian Cancer

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Abstract

Background—Douching was recently reported to be associated with elevated levels of urinary metabolites of endocrine disrupting phthalates, but there is no literature on douching in relation to ovarian cancer. Numerous case-control studies of genital talc use have reported an increased risk of ovarian cancer, but prospective cohort studies have not uniformly confirmed this association. Behavioral correlation between talc use and douching could produce confounding.

Methods—The Sister Study (2003–2009) enrolled and followed 50,884 women in the US and Puerto Rico who had a sister diagnosed with breast cancer. At baseline participants were asked about douching and talc use during the previous 12 months. During follow-up (median of 6.6 years) 154 participants reported a diagnosis of ovarian cancer. We computed adjusted hazard ratios (HR) and 95% confidence intervals (CI) for ovarian cancer risk using the Cox proportional hazards model.

Results—There was little association between baseline perineal talc use and subsequent ovarian cancer (HR: 0.73 CI: 0.44, 1.2). Douching was more common among talc users (OR: 2.1 CI: 2.0, 2.3), and douching at baseline was associated with increased subsequent risk of ovarian cancer (HR: 1.9 CI: 1.2, 2.8).

Conclusions—Douching but not talc use was associated with increased risk of ovarian cancer in the Sister Study.

Keywords

ovarian cancer; talc; douching; phthalates

Introduction

Cancer of the ovary is the most lethal gynecological cancer in women, and its etiologies remain poorly understood. In 2015, there were an estimated 21,290 new cases and 14,180 ovarian cancer deaths among women in the United States (1). Family history of ovarian or breast cancer is a major risk factor. Nulliparity is also associated with increased risk of

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Gonzalez (2016)

- **Overview:** Required participants to have a sister with breast cancer to enroll
- **Participants:** 154 cases, 154 controls (only 17/154 (14%) controls reported talc talc)
- **Data Collection Period:** 2003 through 2009
- **Questionnaire:** Self-Administered
  - Inquired only of individual's Talc usage during the 12 months before enrollment
  - Follow up 6.6 yrs
- **Dose Response Data:**
  - No dose response data / no cumulative lifetime use data
- **Findings:**
  - Talc use w/o adjusting for douching RR: 0.73 (0.44-1.2)
  - Talc use after adjusting for douching RR: 0.70 (0.42-1.1)
  - Douching OR: 1.8 (1.2-2.8)
  - Study suggests OC latency is 15 to 20 years; therefore no adjustment for latency
- **Strengths:** Prospective nature limits recall bias
- **Weaknesses:**
  - Poor confirmation of cancer diagnosis (no medical records for over 1/3 of cases)
  - Did not address dose response
  - Insufficient power to detect RR
  - Exposure misclassification (only collected information on recent/12 months' use)
  - Limited generalizability (all participants had sisters with breast cancer)

Strengths and Limitations: Case-Control Studies

Study	Natl	Cases	Ctrls
Schildkraut 2016	US	584	745
Cramer 2016	US	2041	2100
Wu 2015	US	1791	2391
Kurta 2012	US	902	1802
Rosenblatt 2011	US	812	1313
Wu 2009	US	609	688
Moorman 2009 (white)	US	1114	1086
Moorman 2009 (black)	US	1114	1086
Merritt 2008	Aus	1576	1509
Mills 2004	US	256	1122
Ness 2000	US	767	1367
Cramer 1999	US	563	523
Wong 1999	US	499	735
Godard 1998	Can	170	170
Green 1997	Aus	824	855
Cook 1997	US	313	422
Chang 1997	Can	450	564
Shushan 1996	Isr	200	408
Cramer 1995	US	450	454
Purdie 1995	Aus	824	860
Tzonou 1993	Gre	189	200
Rosenblatt 1992	US	77	46
Chen 1992	Chn	112	224
Harlow 1992	US	235	239
Booth 1989 (daily)	UK	235	451
Booth 1989 (weekly)	UK	235	451
Harlow 1989	US	116	158
Whittemore 1988	US	188	539
Hartge 1983	US	135	171
Cramer 1982	US	215	215

Potential Strengths

- Ascertainment of cases
- Ascertainment of lifetime exposure
- Population based: generalizable
- Sufficient power in many studies

Potential Limitations

- Insufficient power in some
- Exposure misclassification (ascertainment)
- Potential for recall bias
- Potential for confounding

Bradford Hill Guidelines

7 *Section of Occupational Medicine* 295

Meeting January 14 1965

President's Address

The Environment and Disease: Association or Causation?

by Sir Austin Bradford Hill CBE DSC FRCP(hon) FRS
(*Professor Emeritus of Medical Statistics, University of London*)

Amongst the objects of this newly-foun
of Occupational Medicine are firstly 't
means, not readily afforded elsewher
physicians and surgeons with a special
of the relationship between sickness
and conditions of work may discuss
lems, not only with each other, bu
colleagues in other fields, by holding
ings with other Sections of the Society; and,
secondly, 'to make available information about the physical, chemical and psychological hazards of occupation, and in particular about those that are rare or not easily recognized'.

observed *association* to a verdict of *causation*? Upon what basis should we proceed to do so?

I have no wish, nor the skill, to embark upon a philosophical discussion of the meaning of

invariably have to sit around awaiting the results of that research. The whole chain may have to be unravelled or a few links may suffice. It will depend upon circumstances.

Strength	Specificity	Consistency
Temporality	Dose Response	Plausibility
Coherency	Experiment	Analogy

Meta-Analyses and Pooled Studies (All Ovarian)

Study	#Studies	#Cases	DR	RR	CI L	CI U
Taher (2018)[1]	27	17,149	Yes	1.28	1.2	1.37
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Cramer (1999)	14	3834	N/A	1.4	1.2	1.5
Gross (1995)	10[5]	1509	N/A	1.29	1.02	1.63
Harlow (1992)	6	1106	N/A	1.3	1.1	1.6
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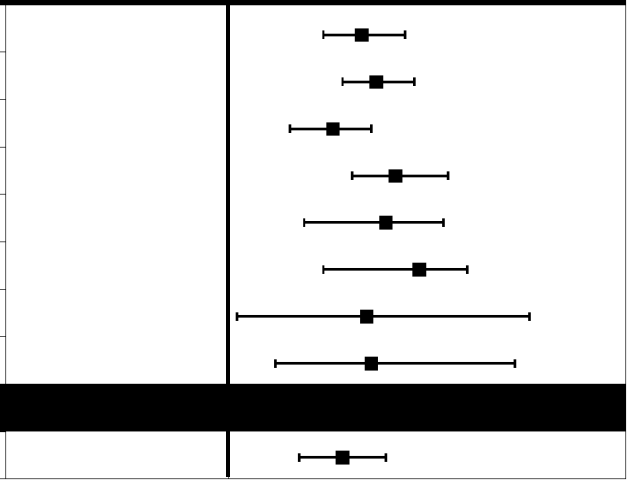

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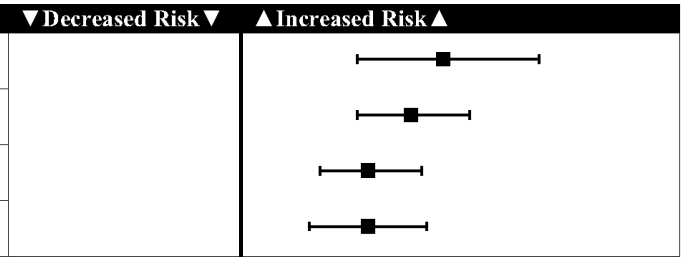

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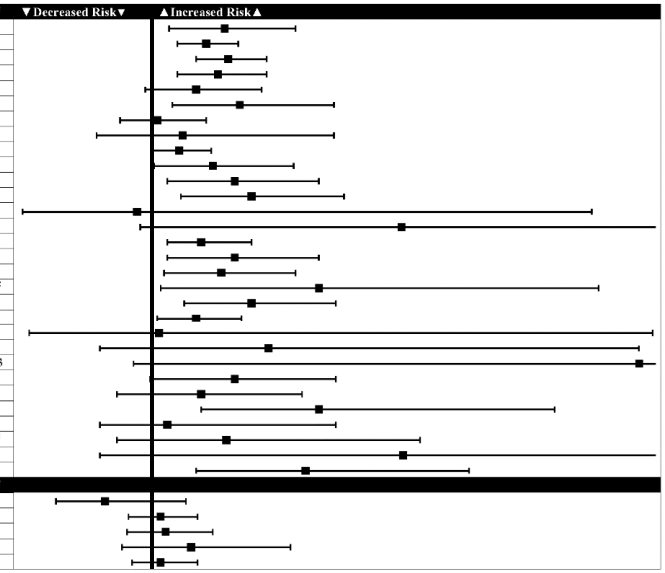
Case-Control and Cohort Studies (All Ovarian)

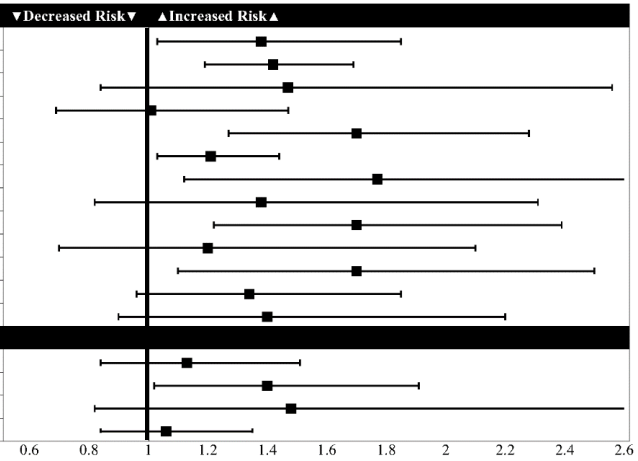
Case-Control Study	Natl	Cases	Ctrls	Pop	DR	RR	CI L	CI U
Schildkraut 2016	US	584	745	Pop.	Yes	1.44	1.11	1.86
Cramer 2016	US	2041	2100	Pop.	Yes	1.33	1.16	1.52
Wu 2015	US	1791	2391	Pop.	Yes	1.46	1.27	1.69
Kurta 2012	US	902	1802	Pop.	N/A	1.4	1.16	1.69
Rosenblatt 2011	US	812	1313	Pop.	No	1.27	0.97	1.66
Wu 2009	US	609	688	Pop.	Yes	1.53	1.13	2.09
Moorman 2009 (white)	US	1114	1086	Pop.	N/A	1.04	0.82	1.33
Moorman 2009 (black)	US	1114	1086	Pop.	N/A	1.19	0.68	2.09
Merritt 2008	Aus	1576	1509	Pop.	Yes	1.17	1.01	1.36
Mills 2004	US	256	1122	Pop.	No	1.37	1.02	1.85
Ness 2000	US	767	1367	Pop.	Incpl	1.5	1.1	2.0
Cramer 1999	US	563	523	Pop.	Yes	1.6	1.18	2.15
Wong 1999	US	499	755	Hosp.	Incpl	0.92	0.24	3.62
Godard 1998	Can	170	170	Pop.	N/A	2.49	0.94	6.58
Green 1997	Aus	824	855	Pop.	Incpl	1.3	1.1	1.6
Cook 1997	US	313	422	Pop.	No	1.5	1.1	2.0[1]
Chang 1997	Can	450	564	Pop.	No	1.42	1.08	1.86
Shushan 1996	Isr	200	408	Pop.	N/A	2	1.06[2]	3.66[2]
Cramer 1995	US	450	454	Pop.	N/A	1.6	1.2	2.1
Purdie 1995	Aus	824	860	Pop.	N/A	1.27	1.04	1.54
Tzonou 1993	Gre	189	200	Hosp.	N/A	1.05	0.28	3.98
Rosenblatt 1992	US	77	46	Hosp.	Yes	1.7	0.7	3.9
Chen 1992	Chn	112	224	Pop.	N/A	3.9	0.9	10.63
Harlow 1992	US	235	239	Pop.	Yes	1.5	1.0	2.1
Booth 1989 (daily)	UK	235	451	Hosp.	Yes	1.3	0.8	1.9[1]
Booth 1989 (weekly)	UK	235	451	Hosp.	Yes	2.0	1.3	3.4
Harlow 1989	US	116	158	Pop.	N/A	1.1	0.7	2.1
Whittemore 1988	US	188	539	H&P	Yes	1.45	0.81[1]	2.60[1]
Hartge 1983	US	135	171	Hosp.	N/A	2.5	0.7	10.0
Cramer 1982	US	215	215	Pop.	N/A	1.92	1.27	2.89
Cohort Studies	**Natl**	**Cases**	**Non**	**Flw Up**	**DR**	**RR**	**CI L**	**CI U**
Gonzalez (2016)	US	154	41,500	6.6	N/A	0.73	0.44	1.2[1]
Houghton (2014)	US	429	61,147	12.4	Incpl	1.06	0.87	1.28
Gertig (2000)	US	307	78,323	N/A	Incpl	1.09	0.86	1.37
Gates (2008)	US	210	600	N/A	Yes	1.24	0.83	1.83
Gates (2010)	US	797	78,323?	N/A	N/A	1.06	0.89	1.28



[1] Corrected data-point from study text (report figure: Cook 1997 CI Upper 2.3; Gonzalez CI Upper 1.21; Booth 1989 CI Upper 1.0; Whittemore CI p=0.06).
[2] Corrected data-point from defense expert report(s) (report figure: p=0.04).

Case-Control and Cohort Studies (Serous)

Study	RR	CI L	CI U
Schildkraut 2016 (serous)	1.38	1.03	1.85
Cramer 2016 (serous)	1.42	1.19	1.69
Rosenblatt 2011 (borderline)	1.47	0.84	2.55[1]
Rosenblatt 2011 (invasive)	1.01	0.69	1.47
Wu 2009 (serous)	1.70	1.27	2.28
Merritt 2008 (serous)	1.21	1.03	1.44
Mills 2004 (serous)	1.77	1.12	2.81[1]
Cramer 1999 (borderline)	1.38	0.82	2.31
Cramer 1999 (invasive)	1.70	1.22	2.39
Wong 1999 (serous)	1.20	0.7	2.1
Cook 1997 (serous)	1.70	1.1	2.5
Chang 1997 (serous)	1.336[1]	0.96	1.85
Harlow 1992 (serous)	1.4	0.9	2.2
Cohort Studies	**RR**	**CI L**	**CI U**
Houghton (2014) (serous)	1.13	0.84	1.51
Gertig (2000) (serous)	1.4	1.02	1.91
Gates (2008) (serous)	1.48	0.82	2.68
Gates (2010) (serous)	1.06	0.84	1.35



[1] Corrected data-point from study text (report figure: Rosenblatt 2011 CI Upper 2.56; Mills 2004 CI Upper 2.80; Chang 1997 RR 1.34).

Mechanism / Biological Plausibility

TALCUM POWDER PRODUCTS → **EXPOSURE** → **MIGRATION** → **INFLAMMATION/ CARCINOGENISIS** → **OVARIAN CANCER**

Containing:

Platy Talc, Fibrous Talc, Asbestos, Heavy Metals and Fragrances

- **Genital Application**
  - Venter (1979)
  - Kunz (1996)
- **Inhalation**
  - IARC (2012)

- **Clinical/Pathology Studies**
  - Henderson (1986)
  - Heller (1996)
  - Venter (1979)
  - Cramer (2007)
  - McDonald (2006)
  - Sjosten (2004)
- **Animal Studies**
- **Regulatory Bodies**
  - Health Canada (2018)
  - FDA (2014)

- **Clinical Studies**
  - Pleurodesis Studies
- **Animal Studies**
  - Keskin (2009)
  - Hamilton (1984)
  - NTP (1993)
- **In Vitro Studies**
  - Shukla (2009)
  - Buz'Zard (2007)
  - Fletcher & Saed (2018)
  - Savant (2018)
- **Scientific Bodies**
  - IOM (2016)
  - Health Canada (2018)
  - IARC (2012)

Dose Response: Terry (2013)

"We evaluated cumulative genital powder exposure as a composite variable of frequency and duration of use. We observed similar increased risks of all nonmucinous subtypes of epithelial ovarian cancer combined across quartiles of genital powder compared with nonuse: ORQ1, 1.18; 95% CI, 1.02–1.36; ORQ2, 1.22; 95% CI, 1.06–1.41; ORQ3, 1.22; 95% CI, 1.06–1.40; ORQ4, 1.37; 95% CI, 1.19 1.58 (Table 5). Although a significant increase in risk with an increasing number of genital powder applications was found for nonmucinous epithelial ovarian cancer when nonusers were included in the analysis (Ptrend < 0.0001), no trend in cumulative use was evident in analyses restricted to everusers of genital powder (Ptrend ¼ 0.17; Table 5). Taken together, these observations suggest that the significant trend test largely reflects the comparison of ever-regular use with never use." (p. 817)

Table 5. Association between estimated lifetime applications of genital powder and risk of ovarian cancer (borderline and invasive combined)

Lifetime number of applications[a]		All cases ($N = 7,587$)		Nonmucinous cases ($N = 6,361$)	
	Controls (%)	Cases (%)	OR[b] (95% CI)	Cases (%)	OR[b] (95% CI)
Never users	6,175 (76)	5,384 (71)	1.00	4,472 (70)	1.00
Quartile 1	509 (6)	534 (7)	1.14 (1.00–1.31)	467 (7)	1.18 (1.02–1.36)
Quartile 2	512 (6)	541 (7)	1.23 (1.08–1.41)	456 (7)	1.22 (1.06–1.41)
Quartile 3	497 (6)	542 (7)	1.22 (1.07–1.40)	457 (7)	1.22 (1.06–1.40)
Quartile 4	486 (6)	586 (8)	1.32 (1.16–1.52)	509 (8)	1.37 (1.19–1.58)
P_{trend}[c]			0.17		0.17

[a]Age-specific 25th, 50th, and 75th percentile cutoff points are 612, 1,872, and 5,400 for participants < 40 years old; 612, 2,160, and 7,200 for 41–50 years; 720, 3,600, and 10,800 for 51–60 years; 1,440, 5,760, and 14,440 for 61–70; 840, 7,200, and 18,000 for > 70 years.
[b]ORs were estimated using conditional logistic regression conditioned on 5-year age groups and adjusted for age (continuous), oral contraceptive duration (never use, <2, 2–<5, 5–<10, or ≥10 years), parity (0, 1, 2, 3, or 4+ children), tubal ligation history (no or yes), BMI (quartiles), race/ethnicity (non-Hispanic White, Hispanic White, Black, Asian, or other).
[c]Trend excludes never users.

(p. 819), *P*trend < .01 versus never users

Dose-Response: Penninkilampi (2018)

ORIGINAL ARTICLE

Perineal Talc Use and Ovarian C

A Systematic Review and Meta-An

Ross Penninkilampi, and Guy D. Eslick

TABLE 1. Summary of Pooled Effect Sizes for Examined Outcome Variables

	No. Studies	Effect Size OR (95% CI)	Heterogeneity I^2	Heterogeneity *P*	Publication Bias *P*
Method of talc use					
Any perineal	26	1.31 (1.24, 1.39)	10.52	0.31	0.09
Any non-perineal	5	1.24 (1.01, 1.51)	66.84	0.02	0.86
Diaphragm	8	0.84 (0.68, 1.05)	14.76	0.31	0.64
Sanitary napkins	12	1.15 (0.94, 1.41)	43.82	0.05	0.17
Length of talc use					
Long-term use (>10 years)	12	1.25 (1.10, 1.43)	45.11	0.04	0.31
<3600 total applications	5	1.32 (1.15, 1.50)	1.83	0.41	0.20
>3600 total applications	5	1.42 (1.25, 1.61)	12.59	0.33	0.40

(p. 44)

Dose-Response: Berge (2018)

Genital use of talc and risk of ovarian cancer: a meta-analysis

Wera Berge[a], Kenneth Mundt[b], Hung Luu[c] and Paolo Boffetta[d]

Some epidemiological studies
between genital use of talc pov
ovarian cancer, but the evidenc
performed a meta-analysis of e
formally evaluate this suspecte
search was conducted in Medli
leading to the identification of
three cohort studies. In the me
random-effect model to calculat
association between genital us
ovarian cancer. We assessed p
study heterogeneity and presen

Table 3 Duration and frequency of use of genital talc – results of meta-analysis

	Number of risk estimates	RR	95% CI
Duration (10 years)	12	1.16	1.07–1.26
Frequency (1 time/week)	7	1.05	1.04–1.07

CI, confidence interval; p-het, RR, relative risk.

(p. 254)

Bradford Hill Guidelines: Findings and Weight

Guideline	Finding	Weight
Strength	Strongly supports a causal association	High Weight
Consistency	Weighs heavily as to the consistency and reliability of the data in favor of a causal risk	Significant Weight
Dose-Response	Findings within the study data, particularly meta-analyses and the pooled analysis, thus, supports my causal analysis	Significant Weight
Plausibility	While this mechanism of carcinogenicity is not proven, it is highly biologically plausible based on the present scientific information and understanding	Significant Weight
Temporality	Finding of temporality is an important component in the causal analysis	Great Weight
Coherence	Cause-and-effect …data on talcum powder product use and risk of ovarian cancer clearly do not significantly conflict with …biology of the disease.. factors support a causal association	Weigh < Strength and Consistency Factors
Specificity	Specificity aspect is present for epithelial ovarian cancer and certain subtypes	Moderate Weight
Analogy	Increased inflammation …associated with increased ovarian cancer risk, and since talc causes an inflammatory response in tissues, this strengthens the link between talcum powder products use and ovarian cancer risk	Weigh < Strength and Consistency Factors
Experiment	While there are experimental data supporting causation from cell studies and animal models… there are no human experimental data. Despite this, data from reliable observational studies … strongly support causation.	Slight Weight

My Opinions

- Published studies establish women who ever used talcum powder products in the genital area had a statistically significant **22-31% increased risk** of developing **ovarian cancer**.
- Evidence of a **dose-response relationship** shows that increasing the amount of exposure to talcum powder products in the genital area results in increasing the risk of developing epithelial ovarian cancer.
- Talcum powder products can **migrate** from the genital area to the ovaries and fallopian tubes.
- Talc has been shown to cause an **inflammatory response** and elevated levels of inflammation in women are associated with increased risk of ovarian cancer.
- All of this provides a **biologically plausible** pathway by which talcum powder product exposure can cause ovarian cancer.
- **Asbestos** is a Class 1 carcinogen found in talcum powder products. The presence of asbestos adds to the evidence of biologic.
- Use of talcum powder products in the genital area **can cause ovarian cancer**.

Exhibit 2

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Review

Critical review of the association between perineal use of talc powder and risk of ovarian cancer

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ABSTRACT

Over the past four decades, there has been increasing concern that perineal use of talc powder, a commonly used personal care product, might be associated with an increased risk of ovarian cancer.

Objectives: To critically review all available human epidemiological data on the relationship between perineal use of talc powder and ovarian cancer, with consideration of other relevant experimental evidence.

Methodology: We identified 30 human studies for qualitative assessment of evidence, including 27 that were retained for further quantitative analysis.

Results: A positive association between perineal use of talc powder and ovarian cancer was found [OR: 1.28 (95% CI: 1.20–1.37)]. A significant risk was noted in Hispanics and Whites, in women applying talc to underwear, in pre-menopausal women and in post-menopausal women receiving hormonal therapy. A negative association was noted with tubal ligation.

Conclusion: Perineal use of talc powder is a possible cause of human ovarian cancer.

1. Introduction

Ovarian cancer is a common gynecologic cancer among women in developed countries, occurring at low rates among young women but increasing with age [1]. The annual incidence rate of ovarian cancer during the period 2005–2009 was 12.7/100,000 women, varying by ethnicity. The majority of ovarian cancers are diagnosed at an advanced stage, with 61% having distant metastases at diagnosis. Hereditary risk factors for ovarian cancer, specifically BRCA1 gene mutations, increase the risk above 35 years of age by about 2–3%.

In recent decades, there has been increasing concern that perineal exposure to talc, a commonly used personal care product, might be associated with an increased risk of ovarian cancer. However, the data describing this association is somewhat inconsistent. Perineal application of talc among women varies by geographic location (Supplementary Material I), with prevalence of use generally higher in Canada, the US and the UK compared to Greece, China and Israel [2].

In order to better characterize the potential ovarian cancer risk associated with perineal use of talc, we conducted a critical review and meta-analysis of peer-reviewed human studies on this issue. We also examined available toxicological (in-vivo and in-vitro) studies, which also shed light on possible biological mechanisms of action that might support the biological plausibility of any observed effects in humans.

2. Materials and methods

2.1. Literature search and identification of relevant human studies

A critical, multi-step search strategy was used to identify relevant studies on talc from multiple bibliographic databases, relevant national and international agencies and other grey literature sources. Specifically, we conducted a critical search for all original studies

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Table 1
Characteristics and overall findings of all included studies (N = 30).

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[a]
Case-control studies						
Booth et al.* (1989), UK [9]	235/451	Range: 20-65 Mean: 52.4 (cases); 51.4 (controls)	Frequency	No trend found	Possible association with > weekly use.	5
Chang and Risch (1997), Canada [10]	450/564	Range: 35-79 Mean: 57.2 (cases); 57.5 (controls)	Ever use Frequency Duration Time of use Type of use Pelvic surgery Histology	Possible exposure-response with frequency and duration of use	Positive association	7
Chen et al.* (1992), China [11]	112/224	Mean: 48.5 (cases); 49.0 (controls)	Ever use;	No trend analysis conducted	Positive association with use > 3 months	6
Cook et al. (1997), USA [12]	313/422	Range: 20-79	Ever use Duration Type of use Histology Lifetime applications	No trend found	Positive association.	7
Cramer et al. (1982), USA [13]	215/215	Range: 18-80 Mean ± SD: 53.2 ± 1.0 (cases); 53.5 ± 1.0 (controls)	Ever use Type of use Pelvic surgery	No trend analysis conducted	Positive association	6
Cramer et al. (2016), USA [14]	2,041/2,100	Range: 18-80	Ever use; Frequency; Duration; Type of use; Histology; Type of powder; Pelvic surgery; Ethnicity; Age at first use; Time since last exposure;	Significant trend for years since exposure, frequency and duration of use, and number of lifetime applications	Positive association	7
Gates et al. (2008), USA [15]	New England Case Control (NECC): 1,175/1,202 Nurses' Health Study (NHS): 210/600	Mean ± SD: 51 ± 13 (NECC); Mean ± SD: 51 ± 8 (NHS)	Ever use; Frequency;	Significant trend for frequency of use	Positive association	7
Godard et al. (1998), Canada [16]	153/152	Mean: 53.7	Ever use; Sporadic/familial	No trend analysis conducted	No association	5
Green et al. (1997), Australia [17]	824/860	Range: 18-79	Ever use; Pelvic surgery;	No trend found	Positive association	7
Harlow et al. (1989), USA [18]	116/158	Range: 20-79	Ever use; Type of use; Type of powder;	No trend analysis conducted	No association	7
Harlow et al. (1992), USA [19]	235/239	Range: 18-76	Ever use; Frequency; Duration; Type of use; Method of use; Histology; Tumor grade; Type of powder; Lifetime applications; Age of first use; Pelvic surgery;	Significant trend for monthly frequency of use	Positive associations in certain subgroups (talc used before 1960, women < 50 years old, women with 1 or 2 live births)	7
Hartge et al. (1983), USA [20]	135/171	Mean: 52.1 (cases); 52.2 (controls)	Ever use;	No trend analysis conducted	No association	5
Kurta et al. (2012), USA [21]	902/1,802	Range: No range reported (age 25+)	Ever use;	No trend analysis conducted	Positive association	6
Langseth & Kjaerheim (2004), Norway [22]	46/179	Not reported	Ever use,	No trend analysis conducted	No association	4
Merritt et al. (2008), Australia [23]	1,576/1,509	Range: 18-79 Mean: 57.8 (cases); 56.4 (controls)	Ever use; Duration; Histology; Pelvic surgery; Age at diagnosis;	No trend found	Positive association strongest for serous and endometrioid subtypes.	7
Mills et al. (2004), USA [24]	249/1,105	Mean ± SD: 56.6 (cases); 55 (controls)	Ever use; Frequency; Duration; Year of first use; Histology; Pelvic surgery; Time of use; Tumor behavior; Cumulative use;	No trend found	Positive association for invasive and serous invasive tumors.	6
Moorman et al. (2009), USA [25]	African-American: 143/189; White 943/868	Range: 20-74	Ever use; Ethnicity;	No trend analysis conducted	No association	6
Ness et al. (2000), USA [26]	767/1,367	Range: 20-69	Ever use; Duration; Method of use;	No trend found	Positive association for any method of use.	6

(*continued on next page*)

Table 1 (*continued*)

Study (Location)	Sample Size (Cases/ Controls or Cases/ Total Cohort)	Age (Years)	Subgroup Analyses	Exposure-Response Assessment	Overall Author Conclusion	NOS[a]
Rosenblatt et al. (1992), USA [27]	77/46 (analyzed)	Range: ≤30 – 80≥	Ever use; Duration; Type of use; Pelvic surgery;	Positive trend for duration of use since tubal ligation	Possible association	4
Rosenblatt et al. (2011), USA [28]	812/1,313	Range: 35-74	Ever use; Lifetime number of applications; Duration; Year of first use; Age of first use; Age of last use; Time of use; Type of use; Histology;	No trend found	Possible association	7
Schildkraut et al. (2016), USA [29]	584/745	Range: 20-79	Ever use; Frequency; Duration; Histology; Lifetime applications; Menopausal status;	Significant trend with frequency and duration of use, and number of lifetime applications	Positive association	8
Tzonou et al. (1993), Greece [30]	189/200	Range: < 70	Ever use;	No trend analysis conducted	No association	5
Whittemore et al. (1988), USA [31]	188/539	Range: 18-74	Ever use; Frequency; Duration; Type of use; Pelvic surgery;	No trend found	Could neither implicate nor exonerate talc as an ovarian carcinogen	4
Wong et al. (1999), USA [32]	462/693	Mean: 54.9	Ever use; Type of use; Duration; Pelvic surgery;	No trend found	No association	4
Wu et al. (2015), USA [33]	1,701/2,391	Range: 18-79	Ever use; Ethnicity;	No trend analysis conducted	Positive association among Hispanics and non-Hispanic whites, but not African Americans.	7
Wu et al. (2009), USA [34]	609/688	Range: 18-74	Ever use; Frequency; Duration; Type of use; Histology; Time of use; Cancer stage;	Significant trend for frequency and duration of use, and number of lifetime applications	Positive association	7
Cohort studies						
Gates et al. (2010) *, USA [35]	797/108,870	Range: 30-55	≥/week vs < 1/week; Histology;	No trend analysis conducted	Possible association that varies by histological subtype. No association with mucinous tumors.	7
Gertig et al. (2000), USA [36]	307/78,630	Range: 30-55 (at cohort entry)	Ever use; Frequency; Histology; Race;	No trend found	Possible association (modest increase for serous invasive subtype)	5
Gonzalez et al. (2016), USA [37]	154/41,654	Range: 35-74 Median: 57.8	Ever use; Time of use;	No trend analysis conducted	No association	6
Houghton et al. (2014), USA [38]	429/61,285	Range: 50-79 Mean: 63.3	Ever use; Duration; Type of use; Histology;	No trend found	No association	7

* Study not included in the meta-analysis because of overlap among the study populations.

[a] Newcastle-Ottawa Scale (NOS) score for each of the listed studies as assessed in our review.

involving human subjects that examined the association of genital/perineal use of talc powder and risk of ovarian cancer, including studies identified in a previous review by Berge et al. [3]. This review followed the PRISMA guidelines, and more specific guidance provided by the Cochrane Collaboration [4] (see Supplementary Material II, III and IV for details on identification of human studies).

Included studies were individually evaluated and scored by two reviewers (MT and NF), as summarized in Table 1 and detailed in Supplementary Material VI and VII. Excluded human studies and reasons for exclusion are shown in Supplementary Material IV. Studies included in previous reviews by both Berge et al. [3] and Penninkilampi et al [5] are compared in Supplementary Material I.

The quality of included studies was assessed using the Newcastle-Ottawa Scale (NOS) [6] as detailed in Supplementary Material V. We used a cut-off point of 7+ stars to represent studies of higher quality (maximum is 9 stars). This cutoff point has been adopted in the literature as indicative of high-quality observational studies [7,8].

2.2. Literature search and identification of relevant non-human studies

We conducted a critical review of non-human studies selected from 3 major bibliographic databases (Medline, EMBASE and Toxline) to identify potentially relevant animal studies on carcinogenic effect of the poorly soluble talc particles following perineal or intravaginal exposure. Studies that focused on any type of cancer, including ovarian cancer, and perineal exposure were considered. All retrieved studies were examined for relevance and reliability. The initial search identified 1165 studies, including, but not limited to, all studies listed in the 2010 IARC report [2]. After level 1 (title and abstract) and level 2 (full text) screening), 51 references were retained for further review. Of those, 15 were considered relevant to this review. Full details of search strategy, inclusion and exclusion criteria, and included studies are given in Supplementary Material VIII, IX and X, respectively.

Studies were classified into one of the following four categories of reliability: 1) reliable without restriction, 2) reliable with restrictions, 3) not reliable and 4) not assignable. Additionally, category (5) is assigned to special studies focusing on pharmacologic or mechanistic investigations.

2.3. Hazard characterization

Epidemiological studies included in the critical review were qualitatively assessed to examine their potential to inform the analysis. Findings from these studies were evaluated with respect to study design, exposure and outcome ascertainment, as well as potential sources of bias and confounding.

In evaluating evidence from animal studies, consideration was given to the form and relevance of the test material, exposure circumstances, animal species/cell system, and health effects studied. Consistency of results among comparable studies and of results in different sexes, species and strains was considered. Evaluation of relevance of studies in laboratory animals to humans was supported by toxicokinetic information in humans and animals, and by mechanistic data from 14 relevant in-vitro studies.

Animal studies were evaluated for evidence on the association between perineal application of talc and ovarian cancer. Additional information on mechanism of action and toxicokinetics obtained from in-vivo and in-vitro studies were used in evaluating biological plausibility of any observed effects.

2.4. Quantitative meta-analysis

We conducted a meta-analysis of the risk of ovarian cancer in relation to perineal use of talc using quantitative risk estimates reported in 27 original studies, comprising three cohort studies and twenty-four case-control studies (included in Table 1). Studies that had analyzed overlapping study populations were assessed on a case-by-case basis for inclusion into the meta-analysis. The level of detail in the reported findings, including sample size and publication date, were considered when deciding which study to include in the case of overlap (Supplementary Material XI).

Maximally adjusted odds ratios (ORs), hazard ratios (HRs) or relative risks (RRs) – measures that are largely comparable because of the relatively low rate of occurrence of ovarian cancer – were extracted from the original studies. Details of the meta-analytic methods are provided in Supplementary Material XI.

3. Results

3.1. Evidence from human studies

The multiple database search for original human studies yielded 656 references. Although a grey literature search yielded another 477 references, only 5 were judged relevant the present analysis. Automatic followed by manual removal of duplicates identified 282 references for screening and review.

Multi-level screening and full-text examination resulted in the in the inclusion of 30 studies for further qualitative/quantitative analyses (Supplementary Materials III and IV). A detailed PRISMA flow diagram is shown in Fig. 1 [9]. Key characteristics of the included 26 case-control studies and four cohort studies are summarized in Table 1. This include study location, sample size, age, performed subgroup analyses, exposure-response assessment, overall conclusion (as reported by the authors, and the Newcastle Ottawa Scale (NOS) score.

Twenty-one of the thirty studies were carried out in the USA, with the remaining studies conducted in Europe (n = 4), Canada (n = 2), Australia (n = 2) and China (n = 1). Forty percent (n = 12) of the studies were relatively recent, published in the last decade, with the remaining studies published between 1982 and 2006. The study populations generally included adult women. Several studies analyzed data from populations initially recruited for other purposes, such as the Nurses' Health Study (NHS) [10–12] and Women's Health Initiative (WHI) [13].

The number of ovarian cancer patients analyzed varied from as few as 46 cases [14] to 22,041 cases per study [10]. Twenty-seven out of the 30 included studies assessed the association between ever use of perineal talc use and ovarian cancer. Subgroup analyses examining the effect of frequency and duration of use, type of use, period of use and other factors varied among these studies (Table 2).

Sixty three percent (n = 19) of the studies concluded the presence of a positive association between perineal exposure to talc powder and ovarian cancer risk [10,11,15–31]. Ten studies concluded the absence of an association [12–14,32–38]. Only one study could not reach a clear conclusion on the presence or absence of an association [39]. Many of the included studies reported variability in some of the analyzed subgroups regarding possible association between exposure to talk powder and risk of ovarian cancer. Supplementary Material VI presents the findings and details of all the studies included in our review, while Supplementary Material VII summarizes the strengths and limitations of each of these studies as identified by the original study authors and by us.

3.2. Evidence from Non-Human studies

After removal of duplicates, the bibliographic database searches on non-human studies initially yielded 1165 references. The 48 retained animal studies focusing on the carcinogenicity of talc, mechanism of action, and toxicokinetics are summarized in Supplementary Material VIII, IX and X.

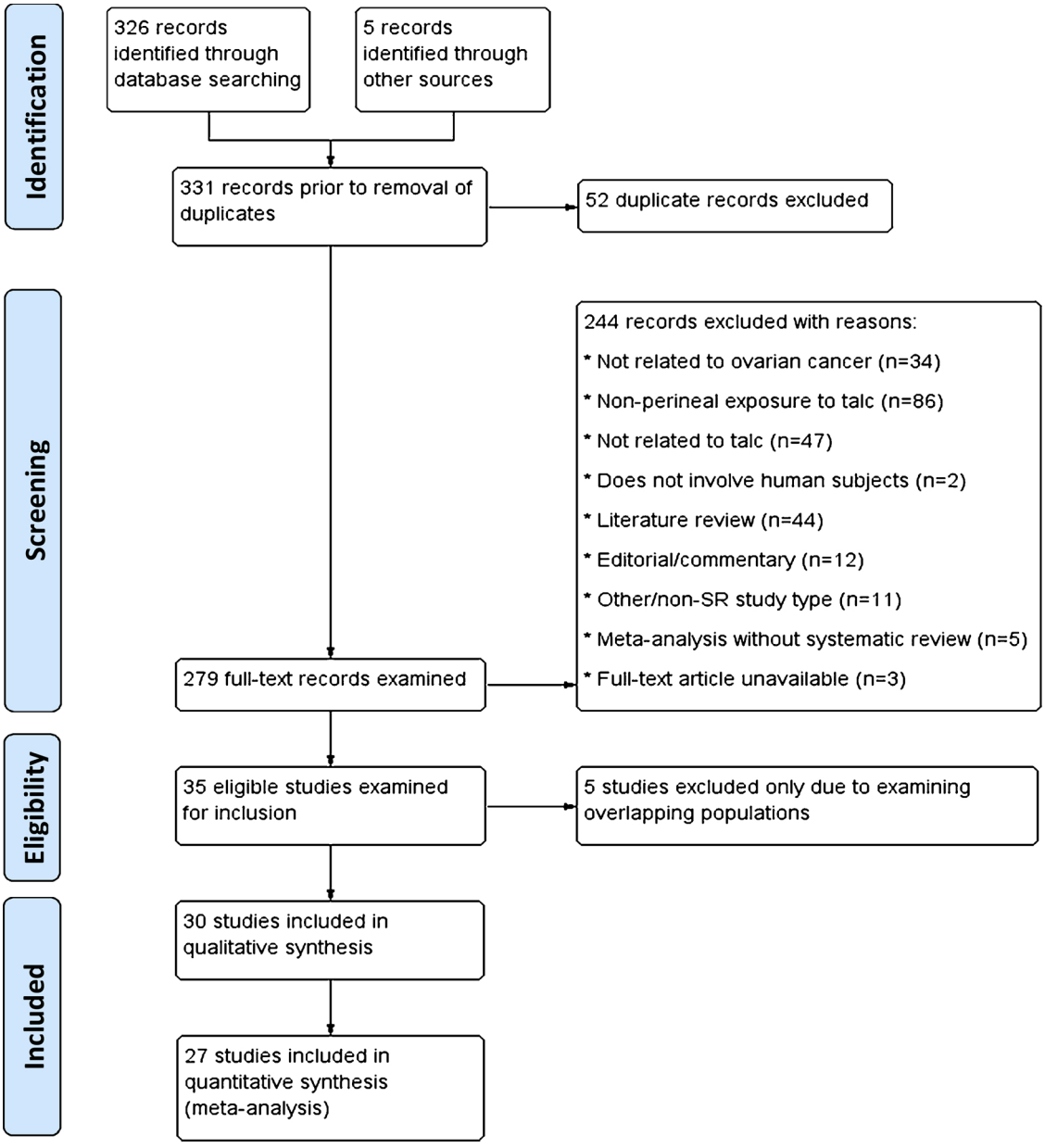

Fig. 1. PRISMA flow diagram.

3.3. Hazard characterization

3.3.1. Evidence from human studies

The case-control studies generally included adult women participants. Cases were commonly selected from registries or hospital records, and included all eligible subjects within a specific geographic region and diagnosed with ovarian cancer within a predetermined time period. Controls were generally matched to cases by age and residence. All the included studies compared the risk of ovarian cancer in ever vs never users of talc (perineal application). However, several of the studies also included subgroup analyses to examine the potential effect of frequency of use, duration of use, tumor histology, ethnicity, method of use, lifetime number of applications, year of first use, and menopausal status. Some authors concluded that the risk of ovarian cancer is limited to [or stronger in] certain subgroups (weekly talc users, premenopausal women) or for specific histology types (notably serous tumors).

Studies reported effect estimates adjusted for a variety of potential confounders (see detailed tables in Supplementary Material VI and VII). Age and parity were considered the two most important variables that could introduce potential bias, based on prior literature: few studies reported findings that were not adjusted for these two variables. As many of the studies only reported on the ovarian cancer risk assessing only one exposure category (comparing only ever vs never users of talc), exposure-response analyses were not done in all studies. When conducted, findings from trend analyses were not consistent.

3.3.2. Evidence from non-human studies

The following aspects were considered in assessment of ovarian cancer and perineal exposure to talc:

- Evidence on ovarian cancer reported in animal studies; and
- Potential hazards arising from the physical and chemical properties of talc, including potential structure-activity relationship indicative of carcinogenic potential;
- The toxicokinetics of talc and the ability to migrate from the perineal area to ovaries and quantity at the actual target site (the tissue dose);
- Findings from in vitro studies suggestive of mechanism of action of carcinogenic effect.

Table 2
Results of the subgroup analysis of talc exposure and ovarian cancer.

Outcome or Subgroup	Studies	Effect Estimate [95% CI]	Heterogeneity I^2 Statistic [p-value]
1 ***Talc use***			
Ever vs. Never	27	1.28 [1.20, 1.37]	33% [< 0.00001]
Ethnicity	3		77% [0.08]
African Americans	3	1.67 [0.90, 3.10]	48% [0.10]
Hispanics	2	1.70 [1.17, 2.47]	0% [0.005]
Whites	3	1.28 [1.11, 1.49]	56% [0.001]
Asians	1	0.04 [0.01, 0.16]	N/A
2 ***Study Assessment***			
●	27		33% [< 0.00001]
○ Study Design			
Case-Control	24	1.32 [1.24, 1.40]	22% [< 0.00001]
Cohort	3	1.06 [0.90, 1.25]	17% [0.49]
●	24		22% [< 0.00001]
○ Type of Controls			
Hospital-based	4	0.96 [0.78, 1.17]	0% [0.66]
Population-based	19	1.34 [1.27, 1.41]	0% [< 0.00001]
Combined	1	1.45 [0.81, 2.60]	N/A
●	27		33% [< 0.00001]
○ Quality Score (NOS)			
NOS > =7	12	1.32 [1.25, 1.40]	0% [< 0.00001]
NOS < 7	15	1.21 [1.05, 1.39]	47% [0.009]
●	27		33% [< 0.00001]
○ Publication Year			
1980-1989	4	1.23 [0.81, 1.88]	66% [0.33]
1990-1999	8	1.30 [1.13, 1.50]	24% [0.0003]
2000-2009	8	1.25 [1.14, 1.37]	18% [< 0.00001]
2010 and beyond	7	1.31 [1.18, 1.45]	44% [< 0.00001]
3 ***Talc Exposure***			
●	7		35% [< 0.00001]
○ Frequency of Use			
Low	5	1.22 [0.96, 1.54]	54% [0.10]
Medium	2	1.22 [0.98, 1.53]	0% [0.08]
High	7	1.39 [1.22, 1.58]	23% [< 0.00001]
●	6		5% [0.0008]
○ Duration of Use			
< 10 Years	5	1.22 [1.03, 1.45]	0% [0.02]
10 - < 20 Years	2	1.42 [1.02, 1.99]	0% [0.04]
20+ Years	2	1.19 [0.71, 1.98]	75% [0.51]
●	13		52% [0.001]
○ Method of Use			
Sanitary Napkin	11	1.12 [0.91, 1.39]	50% [0.29]
Diaphragm	10	0.87 [0.72, 1.05]	25% [0.14]
Underwear	2	1.70 [1.27, 2.28]	0% [0.0004]
Male Condom	3	0.99 [0.73, 1.32]	0% [0.92]
4 ***Tumor Histology***			
●	8		23% [< 0.00001]
○ Tumor Histology			
Serous	7	1.38 [1.22, 1.56]	0% [< 0.00001]
Mucinous	5	1.05 [0.85, 1.29]	23% [0.41]
Endometrioid	6	1.39 [1.05, 1.82]	2% [0.03]
Clear Cell	1	0.63 [0.15, 2.65]	
5 ***Tumor Behavior***			
●	4		0% [< 0.00001]
○ All Grades			
All Invasive	3	1.38 [1.15, 1.65]	0% [0.0004]
All Borderline	4	1.43 [1.08, 1.89]	19% [0.01]
●	5		0% [< 0.00001]
○ Serous			
Serous Invasive	5	1.32 [1.13, 1.54]	24% [0.00004]
Serous Borderline	3	1.39 [1.09, 1.78]	0% [0.008]
●	3		38% [0.40]
○ Mucinous			
Mucinous Invasive	2	1.34 [0.48, 3.79]	70% [0.58]
Mucinous Borderline	3	1.18 [0.76, 1.82]	34% [0.46]
●	1		N/A
○ Endometrioid			
Endometrioid Invasive	1	1.38 [1.06, 1.80]	
●	1		N/A
○ Clear Cell			
Clear Cell Invasive	1	1.01 [0.65, 1.57]	
6 ***Modifiers***			
●	2		78% [0.007]
○ Menopausal State			
Pre-menopausal	2	1.42 [1.16, 1.75]	0% [0.0008]

(*continued on next page*)

Table 2 (*continued*)

Outcome or Subgroup	Studies	Effect Estimate [95% CI]	Heterogeneity I^2 Statistic [p-value]
Post-Menopausal (HT)	2	2.28 [1.72, 3.01]	0% [< 0.00001]
Post-Menopausal (no HT)	2	1.05 [0.84, 1.32]	25% [0.66]
●	7		78% [0.35]
○ Pelvic Surgery			
Tubal Ligation	3	0.64 [0.45, 0.92]	19% [0.02]
Hysterectomy	4	0.89 [0.54, 1.46]	61% [0.65]
Combined	4	1.06 [0.78, 1.42]	61% [0.72]

*NOS: Newcastle-Ottawa Scale for quality scoring of observational studies (maximum is 9 stars).
Low: Once daily for 1 – < 10 days/month; **Medium: Once daily for 10–25 days/month; **High:** Once daily for > 25 days/month.

While the limited data from the animal studies that considered various routes of talc administration are inconsistent [40–45], there are observations from in vivo and in vitro studies which support the potential for local carcinogenic action of talc particles on fallopian, ovarian and peritoneal epithelium [26,46–52].

The results from the *in vitro* studies are informative for mechanisms of action of possible carcinogenicity. Smith and colleagues [53] identified 10 key characteristics (KCs) commonly exhibited by established human carcinogens.

Oxidative stress (KC 6) and inflammation (KC 5) in cell cultures induced by talc have been reported by several authors [47], corresponding to two of the 10 key characteristics (KCs) described by Smith et al. [53]. Several authors suggested additional potential mechanisms of action through cell proliferation (KC 10) and changes in gene expression, presumably facilitated by oxidative stress and dysregulated antioxidant defense mechanisms [48,54].

Chronic perineal or vaginal exposures of animals to talc do not directly affect ovulation or steroidal hormone levels, but can induce chronic local inflammation, which has been suggested as a risk factor for ovarian cancer [55]. Mechanism of action studies suggested that talc can complex iron on the surface and disrupt iron homeostasis, associated with oxidant generation, macrophage distress and leukotriene released by macrophages in the surrounding cells resulting in a chronic inflammatory response which could possibly contribute to tumor promotion in both animals and humans [47,49,50].

The changes seen in cultured cells after exposure to talc particles [49,50] are consistent with those inflammatory and proliferative processes in the lungs seen in laboratory animals after inhalation exposure in a 1993 study conducted by the US National Toxicology Program [46]. In female rats, hyperplasia of alveolar epithelium was associated with inflammatory response and occurred in or near foci of inflammation [46]. The severity of the fibrous granulomatous inflammation in the lungs increased with increased talc concentrations and exposure duration and a significant association was observed between inflammation and fibrosis in the lungs and the incidence of pheochromocytomas in this study [46]. Overall, the available experimental data suggests that long-term irritation, followed by oxidative stress and chronic inflammation, may be involved in local carcinogenic effects of talc in the ovaries.

Local inflammation of the epithelial ovarian surface in rats following by injection of a suspension of talc particles resulted in the development of foreign body granulomas surrounding talc particles and large ovarian bursal cysts [52]. It is generally accepted that benign and malignant ovarian epithelial tumors arise from surface epithelium and its cystic derivatives, and surface epithelial cysts have a greater propensity to undergo neoplasia than does the surface epithelium itself [56]. Evidence of neoplasms of epithelial origin, nuclear atypia, or mitotic activity in the surface epithelium was not found in this study; however, focal areas of papillary changes in the surface epithelium consistent with the histological signs of premalignancy were observed in 40% of treated animals [52].

Structure-activity relationships can provide useful information for assessing potential carcinogenicity. Although structure-activity models predict that poorly soluble particulates such as carbon black and titanium dioxide may be potentially carcinogenic [2], the extension to talc particles is not immediate.

Although inconsistent, there is some evidence that talc particles may migrate in the genital tract of animals [57–60]. Some studies have reported lack of migration of neutron-activated talc from the vagina to the ovaries in cynomolgus monkeys [57], but talc particles were identified in the ovaries of rats that received intrauterine instillation of talc [59]. Radioactivity was not found in the ovaries of rabbits dosed intravaginally with tritium-labelled talc, but was detected in cervix and fallopian tubes [58–60]. Henderson and colleagues [61] examined human tumor tissue of patients with ovarian and cervical tumors, detecting talc particles in histological samples from 10 of 13 ovarian tumors, 12 of 21 cervical tumors, and 5 of 12 normal ovarian tissue samples [61].

Historically, the concern for talc carcinogenicity has been associated with its contamination by asbestos fibers (tremolite) [62], which is considered carcinogenic to humans [2]. In response to this concern, talc, including baby powder, available in the US, contains only U.S. Pharmacopeia (USP) grade pure talc [63]. Talcum powder has been asbestos-free since the 1976 where the specifications for cosmetic talc were developed [64].

3.4. Meta-Analysis

The use of genital talc was associated with a significant increase in the risk of epithelial ovarian cancer, with an overall odds ratio [OR] based on our meta-analysis of 1.28 (95% confidence interval [CI]: 1.20–1.37 P < 0.0001, I^2 = 33%), as presented in Fig. 2. This result is comparable to those of earlier meta-analyses conducted by other investigators [3,5,65–67] as shown in Supplementary Material I.

An increased risk is more apparent in Hispanics and Whites, in women applying talc to underwear, in pre-menopausal women and post-menopausal women receiving hormonal therapy, as well as for the serous and endometrioid types of ovarian cancer (Table 2 and Supplementary Material XI). A negative association was noted with tubal ligation. Our analysis pooled risk estimates from 27 original studies including 3 cohort studies and 24 case-control studies, spanning across four decades (1982–2016) and including a total of 16,005 cases and 201,881 controls from different ethnicities.

In assessing heterogeneity among included studies, most subgroup analyses reported an I^2 statistic ranging between 0%–40%, which will have only a minimal impact on the analysis [4]. Only three subgroup analyses (ethnicity, menopausal state, and pelvic surgery) reported an I^2 statistic of 77%–78%, where considerable heterogeneity might have had an impact on the results [4]. (See Table 2 and Supplementary Material XI for a listing of I^2 statistic values for the different subgroup analyses)

Whereas case-control studies showed a significant increase in the risk of ovarian cancer for “ever vs never” users of talc powder [OR: 1.32 (95% CI: 1.24–1.40), P < 0.00001, I^2 = 22%], cohort studies failed to show a significant increase in risk [OR: 1.06 (95% CI: 0.9–1.25), P = 0.49, I^2 = 17%]. Thirteen out of 24 case-control studies (54%)

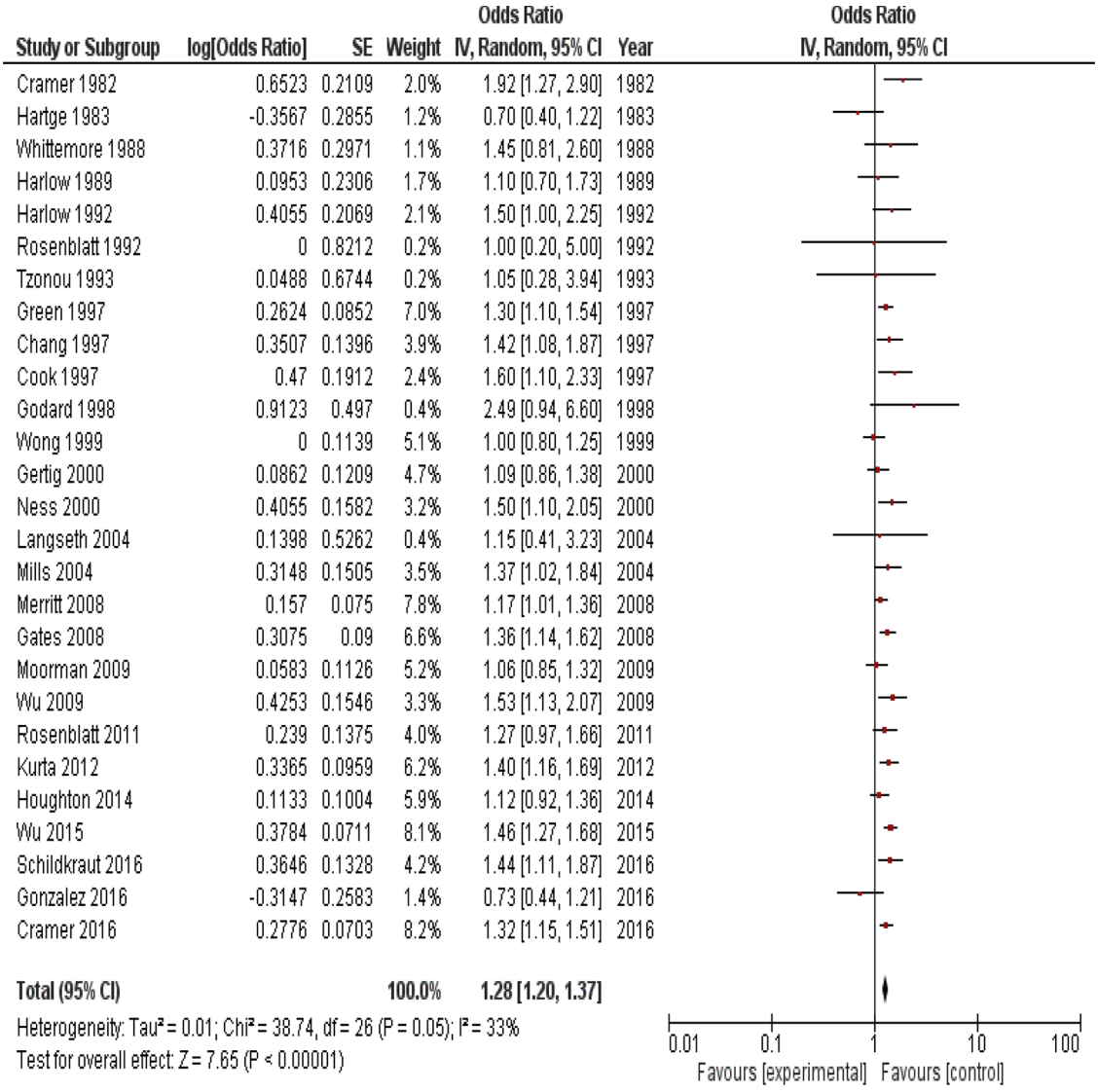

Fig. 2. Forest plot of the meta-analysis results on perineal use of talc and risk of ovarian cancer.

showed a statistically significant association, whereas none of the 3 cohort studies showed a significant overall association between ever vs never genital talc exposure and risk of ovarian cancer.

Subgroup analysis by study quality using the Newcastle Ottawa Scale (NOS ≥ 7 vs NOS < 7) did not show any significant differences in the overall pooled risk estimate. Similarly, there were no differences among subgroup analysis conducted by decade of publication. A significant association was observed for population-based studies [OR: 1.34 (95% CI: 1.27–1.41), P < 0.00001, I^2 = 0%], but not for enlisting hospital-based controls [OR: 0.96 (95% CI: 0.78–1.17), P = 0.66, I^2 = 0%].

We conducted influence analysis to examine the impact of individual studies on the results of our meta-analysis. No appreciable changes were observed regarding the overall association of perineal talc exposure and the risk of ovarian cancer in response to the exclusion of any single study. Detailed results from the influence analysis are provided in Supplementary Material XI.

Subgroup analysis based on ethnicity indicated that Hispanic women using talc showed the most significant increase in risk of ovarian cancer [OR: 1.70 (95% CI: 1.17–2.47), P = 0.005, I^2 = 0%], followed by White women [OR: 1.28 (95% CI: 1.10–1.49], P = 0.001, I^2 = 56%). African-American women showed an elevated, yet non-significant association with ovarian cancer in [OR: 1.67 (95% CI: 0.90–3.10), P = 0.1, I^2 = 48%].

Analyzing exposure by frequency of talc use, talc exposure was stratified into three groups: high (once daily for > 25 days/month), medium (once daily for 10–25 days/month) and low (once daily for 1– < 10 days/month). The OR for the high-use group was higher in the high-use group compared to the other two groups (medium and low-use groups). Duration of talc use was stratified into three groups: < 10 years, 10 – < 20 years, and 20+ years. The overall odds ratio of the < 10 years' group was lower than the OR for the 10 – < 20 years' group. On the other hand, the OR for the 20+ years' group was lower and not statistically significant. However, this OR was based on two studies that showed considerable heterogeneity (I^2 = 75%). Examining the method of application of talc, application to the underwear subgroup had a statistically significant OR, which was the highest among all subgroups. Diaphragm use showed an expected, yet non-significant, negative association with ovarian cancer, which may be due to its action blocking the ascent of talc particles up the reproductive tract.

Pooled risk estimates were statistically significant for two histological types of ovarian cancer: serous tumors [OR: 1.38 (95% CI: 1.22–1.56), P < 0.00001, I^2 = 0%] and endometrioid tumors [OR: 1.39 (95% CI: 1.05–1.82), P = 0.03, I^2 = 2%]. The mucinous type showed a non-significant association [OR: 1.05 (95% CI: 0.85–1.29), P = 0.41, I^2 = 23%], while there were not sufficient studies to examine the other types of ovarian cancers. Regarding tumor behavior, there

was no appreciable difference between invasive [OR: 1.38 (95% CI: 1.15–1.65), P = 0.0004, I^2 = 0%] and borderline [OR: 1.43 (95% CI: 1.08–1.89), P = 0.01, I^2 = 19%] grades of ovarian cancer. Borderline serous tumors showed slightly greater risk [OR: 1.39 (95% CI: 1.09–1.78), P = 0.008, I^2 = 0%] compared to the serous invasive grade [OR: 1.32 (95% CI: 1.13–1.54), P = 0.0004, I^2 = 24%], while both showed a significant association with perineal talc exposure. However, the mucinous tumors showed a non-significant association with talc exposure, with invasive grades being associated with a greater risk [OR: 1.34 (95% CI: 0.48–3.79), P = 0.58, I^2 = 70%] compared to the borderline grade [OR: 1.18 (95% CI: 0.76–1.82), P < 0.46, I^2 = 34%].

Among post-menopausal women, those receiving hormonal therapy showed the greatest risk [OR: 2.28 (95% CI: 1.72–3.01), P < 0.00001, I^2 = 0%], followed by pre-menopausal women [OR: 1.42 (95% CI: 1.16–1.75), P = 0.0008, I^2 = 0%], and then post-menopausal women not receiving hormonal therapy [OR: 1.05 (95% CI: 0.84–1.32), P = 0.66, I^2 = 25%]. This subgroup analysis suggests that hormonal factors, especially estrogens influence the risk of developing ovarian cancer among postmenopausal women who have perineal talc exposure.

Women with prior ligation of the Fallopian tubes showed a significant reduction in risk [OR: 0.64 (95% CI: 0.45 to 0.92), P = 0.02, I^2 = 19%] against ovarian cancer compared to hysterectomy [OR: 0.89 (95% CI: 0.54–1.46), P = 0.65, I^2 = 61%], whereas both surgeries combined showed no effect [OR: 1.06 (95% CI: 0.78–1.42), P = 0.72, I^2 = 61%]. This might be attributed to the fact that tubal ligation is usually performed at an earlier age, thus preventing entry of talc into the reproductive tract earlier and prolonged exposure to talc, compared to hysterectomy that is performed later in life where a higher exposure has already taken place. In a recent meta-analysis [68], the authors reported a negative association of tubal ligation (27 studies) and hysterectomy (15 studies) with the risk of ovarian cancer: this negative association was more apparent in women who had the surgery at an earlier age. A highly plausible mechanism for this association, as suggested by the authors, involves blocking of ascent of agents such as talc to the ovaries.

A summary of results of our meta-analysis is shown in Table 2. Forest plots of all sub-group analyses are provided in Supplementary Material XI.

3.5. Exposure-response assessment

The effect of increasing frequency or duration of perineal use of talc and the risk of ovarian cancer was assessed in the majority of the studies included in this review. Conflicting findings were reported on the nature of the exposure-response relationship: 11 studies concluded that there is no exposure-response, five studies reported a significant positive trend with either frequency or duration of talc use, and two studies concluded that there might be an exposure-response. The remaining twelve studies did not perform or report on trend analyses.

Findings from the seven studies that indicated a potential increased risk of ovarian cancer associated with increasing use of talc are presented in Table 3. The study by Cramer et al. [10] provides the strongest evidence of an exposure-response relationship and could be considered as a key study for exposure-response assessment. The data used in this study were generated from the Nurses' Health Study originally conducted by Belanger et al. [69], a well-designed high quality cohort study of the factors affecting women's health. The results of this study show an increased risk of ovarian cancer at the three highest exposure categories in this study, with the risk at the lowest exposure level [OR: 1.15 (95% CI: 0.89 to 1.47)] being numerically, although not significantly, elevated. Other studies in Table 3 have provided findings in support of an exposure response based on increasing number of talc applications [22,29,31].

In order to permit more direct comparisons of the exposure-response findings from these studies, and whenever the original study data permits, we standardized exposure measurements into talc-years as shown in Fig. 3. Data points were selected from studies after excluding potential data points that are lacking precise information on the level of exposure to talc. The mid-point of the exposure categories in the exposure-response studies was used for exposure-response assessment.

Overall, the graphical results shown in this Fig. 3 suggest a possible increasing trend in ovarian cancer risk with increasing cumulative exposure to talc; however, there is also a high degree of uncertainty surrounding many of the individual risk estimates. (A formal statistical test for trend was not attempted because of the high degree of heterogeneity among studies noted previously in our meta-analysis discussed in Section 3.4)

4. Discussion

The present analysis of the association between perineal use of talc powder and ovarian cancer risk considered four decades of scientific work exploring the epidemiological associations and non-human studies. The motivation for this review is based on two questions: what do human epidemiology studies of perineal talc exposure reveal about potential ovarian carcinogenicity, and what do in-vitro and in-vivo studies suggest about potential mechanisms of toxicity?

A critical review of the human epidemiology studies was conducted to address the first question. Thirty observational epidemiologic studies were identified and assessed for quality using the NOS [6]. In parallel with the review of human epidemiological evidence, a critical review of evidence exploring in- vivo and in-vitro toxicology data on talc was conducted. Although animal studies have limited relevance to the investigation of carcinogenicity of talc following perineal exposure, experimental evidence from both animal and in vitro studies can accurately represent the cellular and molecular changes associated with the initiation and progression of human ovarian cancer following perineal exposure to talc.

The available animal evidence provides some insights concerning possible mechanisms of talc toxicity, including oxidative stress, immune system alterations and inflammatory responses. However, it also indicates that talc is not genotoxic. In total, the epidemiology studies suggest that perineal exposure to talc powder is a possible human ovarian carcinogen but there are concerns that the actual exposure experienced by these women over the past 40–50 years is not well understood. As reported by Langesth and colleagues [65], there had been some concern that asbestos-contaminated talc powder that was produced prior to 1976 might have been a confounder; however, the similarity of findings between studies published prior to and after this point suggests asbestos contamination does not explain the positive association between perineal use of talc powder and risk of ovarian cancer [25,26].

Human observational studies have inherent limitations that could bias the findings. Potentially important sources of bias reported in the included studies include: 1) selection bias due to low response rates from cases and controls or from limiting subjects to English-speaking women of two specific races, and 2) exposure misclassification due to recall bias inherent in case control studies. Other limitations included small sample sizes in some studies, small numbers of subjects in subgroup analyses, lack of information on duration of talc use in many studies that only compared ever vs never users, as well as lack of information on the talc content of the different brands of genital powders used. In two of the three cohort studies, the follow-up period between exposure assessment and end of study could have been inadequate to detect a potential association between talc exposure and ovarian cancer. Houghton et al. [13] reported a mean follow up of 12.4 years, while Gates et al. [11] followed a cohort of women for 24 years. However, Gertig et al. [12] and Gonzalez et al. [38] noted that one of their main limitations is the relatively short follow up periods that may not be adequate to detect a potential association between talc exposure and ovarian cancer. For example, studies of smoking and ovarian cancer

Table 3
Summary of studies that reported ORs for increasing number of lifetime perineal talc applications.

Study	Stratification	Reported Exposure-Response Strata	aOR*	95% CI
Schildkraut et al. (2016) [29]	Lifetime genital powder applications	< 3600 applications, any genital use vs (never use)	1.16	[0.83, 1.63]
		> 3600 applications, any genital use vs (never use)	1.67	[1.23, 2.26]
Whittemore et al. (1988) [31]	Overall trend	Overall trend for 30 uses per month	1.3	[0.88, 1.92]
Wu et al. (2009) [34]	By total times of talc use	≤ 5200 times vs nonuse	1.2	[0.77, 1.88]
		5201 – 15,600 times vs nonuse	1.38	[0.87, 2.20]
		15,601 – 52,000 times vs nonuse	1.34	[0.89, 2.02]
		> 52,000 times	1.99	[1.34, 2.96]
Mills et al. (2004) [24]	By cumulative use (frequency × duration)	First quartile (lowest exposure)	1.03	[0.59, 1.80]
		Second quartile	1.81	[1.10, 2.97]
		Third quartile	1.74	[1.11, 2.73]
		Fourth quartile (highest exposure)	1.06	[0.62, 1.83]
Rosenblatt et al. (2011) [28]	By lifetime number of applications of perineal powder after bathing	1-1,599 applications	1.21	[0.71, 2.06]
		1,600-4,799 applications	2.08	[1.32, 3.27]
		4,800-9,999 applications	0.87	[0.50, 1.53]
		≥10,000 applications	0.87	[0.48, 1.57]
Cramer et al. (2016) [14]	By total genital applications	≤360 total genital applications	1.15	[0.89, 1.47]
		361-1,800 total genital applications	1.36	[1.06, 1.75]
		1,801-7200 total genital applications	1.41	[1.10, 1.80]
		> 7200 total genital applications	1.39	[1.11, 1.75]
Harlow et al. (1992) [19]	Total Lifetime Perineal Applications**	< 1000 applications	1.3	[0.7, 2.7]
		1000 - 10,000 applications	1.5	[0.9, 2.4]
		> 10,000 applications	1.8	[1.0, 3.0]

* aOR: adjusted odds ratio.
** 10,000 applications are equivalent to daily use for 30 year.

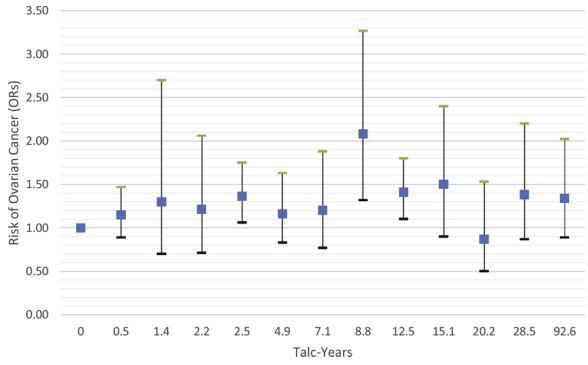

Fig. 3. Ovarian cancer risk estimates at increasing levels of exposure to talc, as reported from multiple studies.

suggest that follow-up periods as long as four decades improve recognition of the carcinogenic effects of smoking [70] longer follow up periods may also improve characterization of the association between talc and ovarian cancer. In this regard, the minimum latency period for radiation-induced ovarian cancer among Hiroshima atomic bomb survivors has been reported to range from 15 to 20 years [71,72]. Common strengths reported in most studies were the selection of population controls in many of the case control studies and having relatively large sample sizes that allowed a multitude of stratified analyses.

Effect estimates in this meta-analysis were pooled from 24 case control studies and 3 cohort studies, and refer to ever vs never use of perineal talc. Pooling by study design showed a notably higher risk estimate for case-control [OR: 1.32 (95% CI: 1.24 to 1.40), P < 0.00001, I^2 = 22%] compared to cohort studies [OR: 1.06 (95% CI: 0.9 to 1.25), P = 0.49, I^2 = 17%]. Although the reasons for this are unclear, the difference could potentially be due to issues relating to latency, study power, or exposure misclassification.

Although cohort study designs are efficient for examining diseases with a long latency period, it is essential that the period between talc exposure and the cancer diagnosis be sufficiently long. Gonzalez et al. [38] suggested that the latency period for ovarian cancer is between 15–20 years. In the cohort studies included in this review,

In addition, cohort studies included may have been underpowered to detect an odds ratio (relative risk) of 1.3 estimated from the case control studies. This was noted by Narod et al. [73], who suggest that cohorts of at least 200,000 women would be needed to reach statistical significance if the true odds ratio is 1.3. The cohort studies included in this review included much smaller cohort sizes, ranging between 41,654 and 78,630 women.

Finally, in cohort studies, talc exposure was assessed at cohort entry and was used as a measure of chronic talc use during follow up. It is possible that women who were not exposed to perineal talc at the time of cohort entry began using talc at a later time, and vice versa, possibly introducing non-differential misclassification of exposure, which could bias the risk estimate towards the null value of unity. Conversely, in the presence of differential exposure misclassification, a bias away from the null hypothesis could accentuate differences between the cohort and case-control studies.

4.1. Exposures and outcomes

All epidemiological studies included in our review evaluated the association between the perineal application of talc and subsequent diagnosis of ovarian cancer. Perineal vs body exposure is an important distinction, as the movement of talc is thought to follow an ascending path from the perineum through the vagina, uterus and fallopian tubes to the ovarian (as well as fallopian tube and peritoneal) epithelium.

Ovarian cancer is a common gynecologic malignancy in developed and developing countries. Risk factors for ovarian cancer include age, infertility, nulligravidity, endometriosis, hereditary ovarian cancer, tobacco and asbestos.

Protective factors for ovarian cancer include oral contraceptives, bilateral tubal ligation, salpingo-oophorectomy, hysterectomy, and breast feeding [74]. It is a difficult cancer to diagnose early, with approximately 60% of the individuals diagnosed after the cancer has metastasized from the pelvic region, where this cancer begins. In the meta-analysis, comparing ovarian cancer risk among women who used talc versus those who never used talc (using both case-control and cohort designs), we observed an approximate 30% increase in ovarian cancer risk in the group who used talc. The most common type of ovarian cancer seen in the general population, and among the women exposed to talc were of epithelial origin, most common histologic type (accounting for about 95% of all cases in the general population), and of serous morphology, the most common subtype (comprising about

75% in the general population).

The cell-type of origin and morphology of talc induced ovarian cancer is similar to that observed in typical ovarian cancer with approximately 95% derived from epithelium (from fallopian tube fimbriae, ovarian or peritoneal) with serous tumors as the most common subtype. Like most ovarian cancers, those associated with talc exposure are typically diagnosed late in the course of the disease (˜60% are diagnosed after the disease has spread outside of the pelvis). This late diagnosis complicates our understanding of the history and origin of the disease.

Demographic factors were analyzed using subgroup analysis where possible, and these were generally consistent with what has been previously observed with respect to ethnicity and risk of ovarian cancer. Additionally, these data also provide support for a mechanism of ovarian cancer induction working via an inflammatory pathway associated with oxidative stress [26,75,76].

A small number of studies explored the issue of ethnicity: Asians (1 study), Hispanics (2 studies), and African-Americans and Whites (3 studies each). Among these studies the risk for talc associated ovarian cancer was 1.70 (Hispanics), 1.67 (African Americans), 1.28 (Whites) and 0.04 (Asians). These risk factors compare with the demographics of ovarian cancer in the US population with an overall prevalence of ovarian cancer of 12.7/100,000 among Whites 13.4/100,00, Hispanics 11.3/100,000, African Americans 9.8/100,000, and Asians 9.8/100,000. The difference in US prevalence and risk of talc induced ovarian cancer among Hispanics and African Americans may provide further evidence concerning exposures or mechanism of action [74].

A variety of factors were assessed with respect to the studies contributing to the meta-analysis, including NOS score for study quality and publication year. In general, the risk of talc associated ovarian cancer was similar among studies with an NOS ≥ 7 or NOS < 7 (maximum is 9). Year of publication also failed to demonstrate a significant impact on reported talc risk estimates.

4.2. Exposure metrics

Given that the epidemiological studies indicate that talc is a possible human carcinogen, we next evaluated the studies to identify those comparing differences in exposure. The initial assessment exploring frequency of use, utilized a qualitative exposure metric: low, medium and high. Ovarian cancer was observed to increase between the medium and high exposure groups, consistent with an exposure-response relationship. Several studies explored duration of use (years) and risk of ovarian cancer; 20+ years (2 studies),10 (5 studies), 10/20 (2 studies), and observed that the risk was greatest in the 20+ year exposure group, followed by lower risk in the 10/20 year and < 10-year exposure groups.

Several studies explored the route of exposure or approach to talc application on ovarian cancer risk, including; hysterectomy, bilateral tubal ligation, diaphragm, underwear, sanitary napkin, as these can provide insight into differences in exposure of the fallopian tube, ovarian and peritoneal epithelium. Use of a diaphragm, as well as tubal ligation act to interrupt exposure of perineal talc to reproductive tract. In contrast, application to underwear and sanitary napkin exposure will provide broader exposures. As hypothesized, the use of diaphragm and bilateral tubal ligation decreased ovarian cancer risk [23].

4.3. Modifying factors

Modifiers of the risk of ovarian cancer, either associated with talc exposure, or a spontaneous disease, can provide clues to potential mechanisms of causation. Menopausal status and use of hormones can modify the risk for ovarian cancer. For example, among post-menopausal women receiving hormonal therapy the risk for ovarian cancer is greater than those who are premenopausal and those who are post-menopausal not receiving hormone therapy. It has also been observed that women receiving fertility treatment who do not become pregnant are at greater risk for ovarian cancer [23]. These data suggest that hormonal status (elevated estrogens and/or gonadotropins) plays a role in the mechanism of action of talc associated ovarian cancer.

Subgroup analyses in the meta-analysis indicated that interruption of the pathway from perineum to pelvis (as with bilateral tubal ligation or use of diaphragm) decreased risk for ovarian cancer. This supports the hypothesis that talc acts locally on the ovary. Evidence from non-human studies suggesting an inflammatory response of epithelial cells to talc, and histological data corroborating those observations, provides additional support for an inflammatory pathway leading to ovarian cancer. One study recently explored the use of anti-inflammatory drugs and observed a decreased risk for ovarian cancer, also supporting an inflammatory pathway due to oxidative stress as a plausible biological mechanism of talc carcinogenicity [75].

4.4. Applying GRADE framework

We applied the GRADE framework [77] to assess the quality of the evidence derived from the studies included this review (Table 4). Using GRADEpro for the assessment, the certainty of the evidence was classified as very low. Several factors are taken into account in the GRADE process. First, we considered our findings from the meta-analysis to lack any serious issues with respect to inconsistency, indirectness, and imprecision. However, we deemed the findings to be subject to an appreciable risk of bias, mainly due to the potential for recall bias in the included case control studies and the relatively short follow-up periods between exposure and outcome assessment in the included cohort

Table 4
GRADE Pro Summary of Findings for Human Studies[a].

Outcomes	Anticipated absolute effects[b] (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)
	Risk with non-use	Risk with perineal use of talc			
Ovarian cancer	64 per 1000	**80 per** 1000 (75 to 85)	**OR 1.28** (1.20 to 1.37)	15,303 cases 199,144 controls (27 observational studies)	VERY LOW[c,d]

[a] **GRADE Working Group grades of evidence are: high certainty (**"We are very confident that the true effect lies close to that of the estimate of the effect."); moderate certainty (We are very confident that the true effect lies close to that of the estimate of the effect.t"); low certainty (Our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect."; and very low certainty ("We have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.).

[b] The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval; **OR:** Odds ratio.

[c] Twenty-four studies were case-control studies; recall bias may be an issue given long latency period.

[d] Three studies were cohort studies, and were assessed as having a relatively short follow-up period for the development of ovarian cancer (15–20 years).

studies.

Study design is a critical component in the GRADE assessment, where randomized controlled trials (RCTs) are viewed as providing considerably stronger evidence than observational studies [77]. As such, the evidence derived from the observational studies in this review was initially classified as being of low certainty within the GRADE framework; this was further downgraded to very low certainty in light of the risk of bias noted above. Despite the very low certainty assigned by the GRADE evaluation, which heavily favors evidence from RCTs (a difficult approach to study the potential carcinogenicity of talc following perineal exposure), we maintain our conclusion that talc is a possible cause of human cancer in humans based on the totality of evidence from multiple observational studies and a plausible biological pathway involving chronic inflammation and oxidative stress.

5. Conclusion

We conducted an extensive search, examination, assessment and analysis of evidence from published original human and non-human studies and from published reviews that considered the association between genital/perineal use of talc powder and risk of ovarian cancer. The steps followed in conducting this review are summarized in Fig. 4, along with the key findings at each step. Consistent with a previous evaluation by the IARC in 2010 [2], the present evaluation of all currently available relevant data indicates that perineal exposure to talc powder is a possible cause of ovarian cancer in humans.

While acknowledging the valuable contributions made by previous research groups, our review provides the most up-to-date and comprehensive examination of the association between perineal exposure to talc and ovarian cancer risk, supported by careful examination of data from the original studies and elimination of studies reporting on overlapping populations. It is reassuring that earlier expert reviews, including the two recent systematic reviews [3,5] arrived at compatible conclusions, thereby reinforcing the robustness of the association between perineal exposure to talc and ovarian cancer risk.

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All authors who contributed to both this study and manuscript report no conflict of interest in relation to the planning for and conducting this study as well as the preparation of this manuscript. Although the research project and manuscript preparation were conducted under contract to Health Canada, the views and conclusions presented in this article are those of the authors alone.

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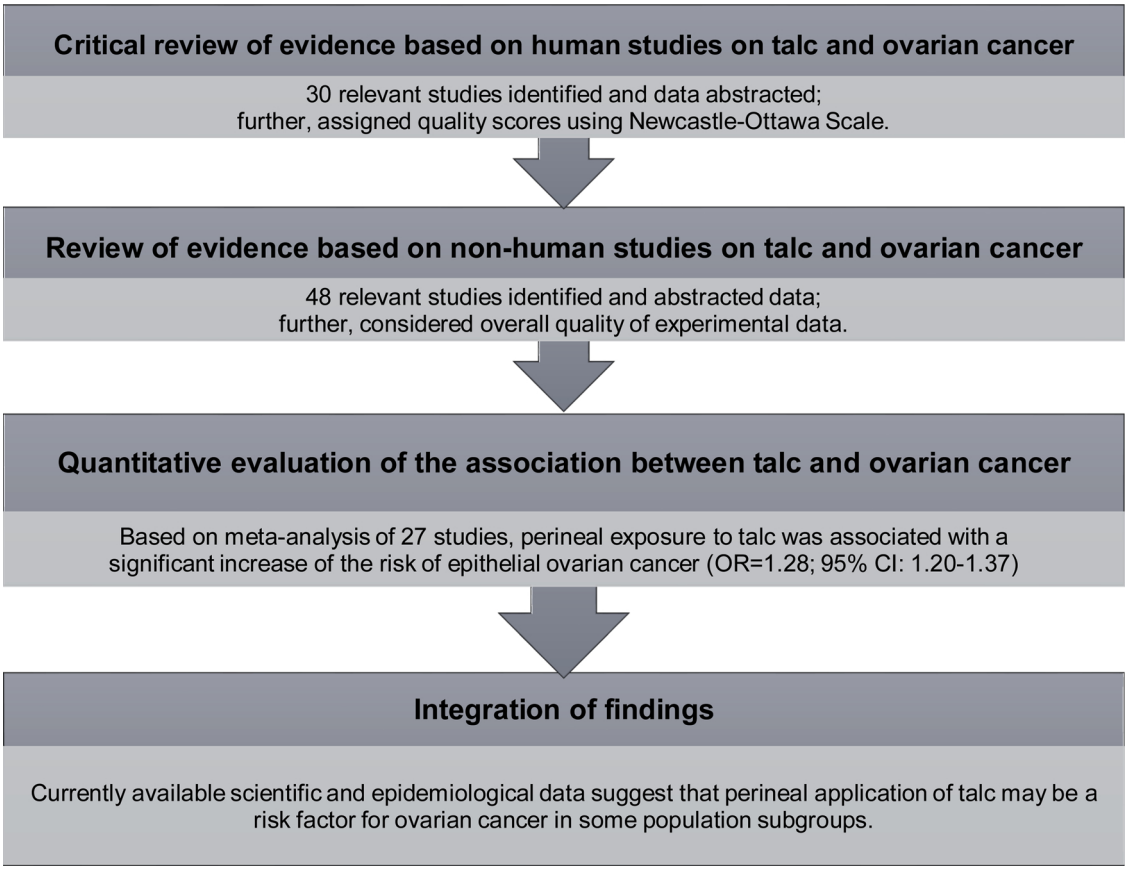

Fig. 4. Detailed process flow for assessment of talc carcinogenecity.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.reprotox.2019.08.015.

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Exhibit 3

Daniel Clarke-Pearson, MD

- Certified by American Board of Obstetrics and Gynecology
- Board certified as sub-specialist in gynecologic oncology
- BA, Harvard; MD, Case Western Reserve
- Residency and Fellowship in gynecologic oncology, Duke University Medical Center
- Division and Fellowship Director GYN Oncology, Duke
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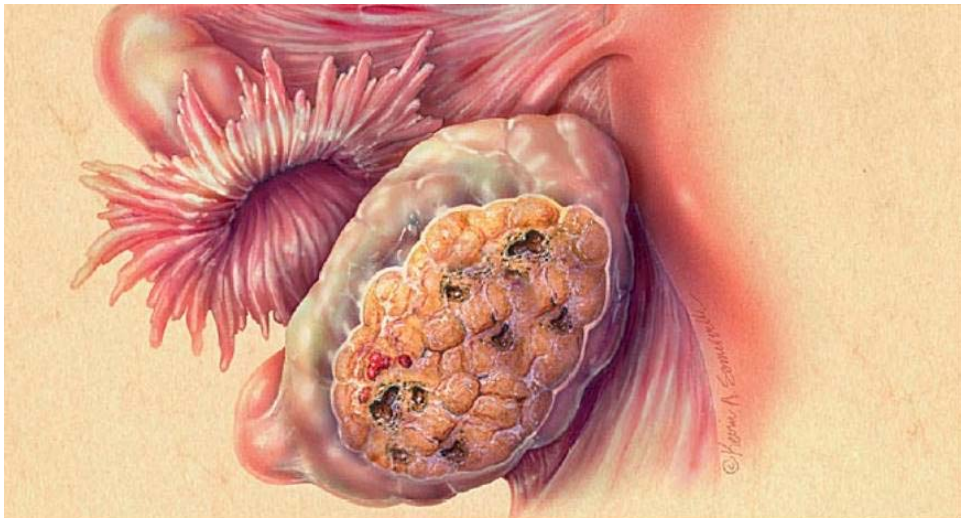
Daniel Clarke-Pearson, MD

- Past President Society of Gynecologic Oncology (SGO)
- Chair of Gynecologic Management Committee, American College of Obstetrics and Gynecology (ACOG)
- Current member of SGO Ethics Committee
- President of Council of University Chairs of Ob GYN
- Authored over 250 peer-reviewed manuscripts and 50 book chapters
- Editorial board of *Obstetrics and Gynecology* and *Gynecologic Oncology*

UNC MEDICAL CENTER
UNC HEALTH CARE

2

Ovarian Cancer



3

Summary of Opinions

- Genital application of talcum powder such as Johnson's Baby Powder and Shower to Shower increases the risk of epithelial ovarian cancer (EOC) in all women and can cause EOC in some women.
- When looking at the epidemiologic studies in their totality, the data shows a consistent, statistically significant increased risk of developing EOC with perineal talcum powder use. Overall, the risk is increased 20-60% when compared with women who did not use talcum powder.
- The ascension of talcum powder and its constituents through the genital tract is the most important route of exposure. Inhalation is another plausible mechanism.
- There is a clear link between inflammation (resulting in oxidative stress) and ovarian cancer risk. Talcum powder is known to elicit an inflammatory response in animals and humans.
- Johnson's Baby Powder and Shower to Shower contain multiple carcinogens including asbestos, fibrous talc, platy talc and heavy metals. Fragrance chemicals also contribute to the carcinogenicity of the product.

4

Methodology

Can the use of talcum powder in the perineal area cause epithelial ovarian cancer and if so, what is the biological mechanism for this occurrence?

Step 1: Review of the epidemiologic literature

Step 2: Review of mechanistic evidence, including migration/inhalation and inflammation

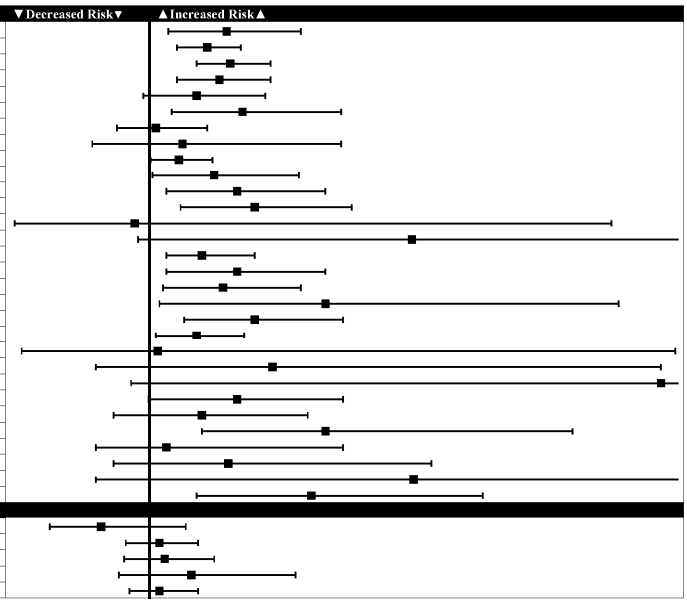
Step 3: Assessment of the use of talcum powder as a risk factor for ovarian cancer

Step 4: Bradford Hill causation analysis

5

Case-Control and Cohort Studies (All Ovarian) (1982-2016)

Case-Control Study	Natl	Cases	Ctrls	Pop	DR	RR	CI L	CI U
Schildkraut 2016	US	584	745	Pop.	Yes	1.44	1.11	1.86
Cramer 2016	US	2041	2100	Pop.	Yes	1.33	1.16	1.52
Wu 2015	US	1791	2391	Pop.	Yes	1.46	1.27	1.69
Kurta 2012	US	902	1802	Pop.	N/A	1.4	1.16	1.69
Rosenblatt 2011	US	812	1313	Pop.	No	1.27	0.97	1.66
Wu 2009	US	609	688	Pop.	Yes	1.53	1.13	2.09
Moorman 2009 (white)	US	1114	1086	Pop.	N/A	1.04	0.82	1.33
Moorman 2009 (black)	US	1114	1086	Pop.	N/A	1.19	0.68	2.09
Merritt 2008	Aus	1576	1509	Pop.	Yes	1.17	1.01	1.36
Mills 2004	US	256	1122	Pop.	No	1.37	1.02	1.85
Ness 2000	US	767	1367	Pop.	Incpl	1.5	1.1	2.0
Cramer 1999	US	563	523	Pop.	Yes	1.6	1.18	2.15
Wong 1999	US	499	755	Hosp.	Incpl	0.92	0.24	3.62
Godard 1998	Can	170	170	Pop.	N/A	2.49	0.94	6.58
Green 1997	Aus	824	855	Pop.	Incpl	1.3	1.1	1.6
Cook 1997	US	313	422	Pop.	No	1.5	1.1	2.0[1]
Chang 1997	Can	450	564	Pop.	No	1.42	1.08	1.86
Shushan 1996	Isr	200	408	Pop.	N/A	2	1.06[2]	3.66[2]
Cramer 1995	US	450	454	Pop.	N/A	1.6	1.2	2.1
Purdie 1995	Aus	824	860	Pop.	N/A	1.27	1.04	1.54
Tzonou 1993	Gre	189	200	Hosp.	N/A	1.05	0.28	3.98
Rosenblatt 1992	US	77	46	Hosp.	Yes	1.7	0.7	3.9
Chen 1992	Chn	112	224	Pop.	N/A	3.9	0.9	10.63
Harlow 1992	US	235	239	Pop.	Yes	1.5	1.0	2.1
Booth 1989 (daily)	UK	235	451	Hosp.	Yes	1.3	0.8	1.9[1]
Booth 1989 (weekly)	UK	235	451	Hosp.	Yes	2.0	1.3	3.4
Harlow 1989	US	116	158	Pop.	N/A	1.1	0.7	2.1
Whittemore 1988	US	188	539	H&P	Yes	1.45	0.81[1]	2.60[1]
Hartge 1983	US	135	171	Hosp.	N/A	2.5	0.7	10.0
Cramer 1982	US	215	215	Pop.	N/A	1.92	1.27	2.89
Cohort Studies	**Natl**	**Cases**	**Non**	**Flw Up**	**DR**	**RR**	**CI L**	**CI U**
Gonzalez (2016)	US	154	41,500	6.6	N/A	0.73	0.44	1.2[1]
Houghton (2014)	US	429	61,147	12.4	Incpl	1.06	0.87	1.28
Gertig (2000)	US	307	78,323	N/A	Incpl	1.09	0.86	1.37
Gates (2008)	US	210	600	N/A	Yes	1.24	0.83	1.83
Gates (2010)	US	797	78,323?	N/A	N/A	1.06	0.89	1.28

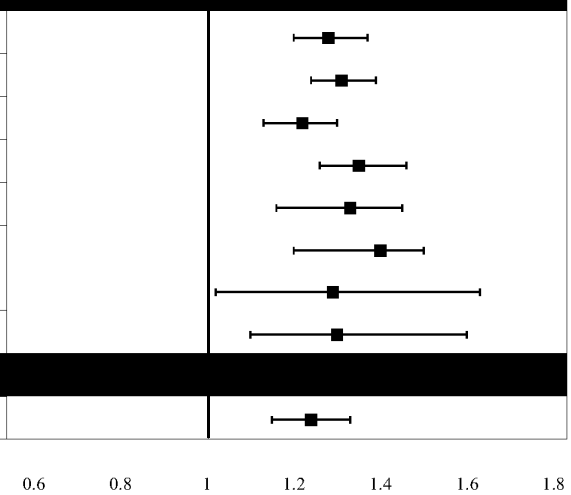


[1] Corrected data-point from study text (report figure: Cook 1997 CI Upper 2.3; Gonzalez CI Upper 1.21; Booth 1989 CI Upper 1.0; Whittemore CI p=0.06).
[2] Corrected data-point from defense expert report(s) (report figure: p=0.04).

6

Meta-Analyses and Pooled Studies (All Ovarian)

Study	#Studies	#Cases	DR	RR	CI L	CI U
Taher (2018)[1]	27	17,149	Yes	1.28	1.2	1.37
Penninkilampi (2018)	27	14,311	Yes	1.31	1.24	1.39
Berge (2018)[2]	27	N/A[3]	Yes	1.22	1.13	1.3
Langseth (2008)	20	N/A[3]	N/A	1.35	1.26	1.46
Huncharek (2003)	16	5260	No[4]	1.33	1.16	1.45
Cramer (1999)	14	3834	N/A	1.4	1.2	1.5
Gross (1995)	10[5]	1509	N/A	1.29	1.02	1.63
Harlow (1992)	6	1106	N/A	1.3	1.1	1.6
Pooled	**#Studies**	**#Cases**	**DR**	**RR**	**CI L**	**CI U**
Terry (2013)	8	8,525	Yes	1.24	1.15	1.33



[1] Taher was published after expert report completed.
[2] Updating to reflect 2018 version of same 2017 study cited in report.
[3] Number of cases not provided.
[4] No summary estimates calculated. Dose response addressed in 9/16 source studies: no dose-response apparent.
[5] N=5 studies with adjusted data and limited to epithelial ovarian cancers.

7

Bradford Hill Analysis

Factor	Analysis	Weight
Strength of Association & Consistency	This opinion is supported by overwhelming epidemiologic evidence showing that the use of talcum powder statistically increases a woman's risk of developing EOC by approximately 30 percent (Odds ratio 1.31; Penninkilampi 2018). Every meta-analysis before 2018 also reported similar increase in the risk of developing EOC with the use of talcum powder.	**Critically important**
Specificity	Based on the epidemiologic studies cited in this report, there appears to be a specific ovarian cancer caused by talcum powder: epithelial ovarian cancer (EOC). This association satisfies this consideration.	**Not as important as strength and consistency**
Temporality	In the case of talcum powder and ovarian cancer there is a clear latency period of decades of talcum powder use before a woman develops ovarian cancer, thus fulfilling this consideration.	**Satisfied**
Biologic Gradient/Dose Response	A number of studies have demonstrated an association between "dose" and the occurrence of EOC (response). (Terry et al. 2013; Schildkraut et al. 2016; Daniel W. Cramer et al. 2016; Penninkilampi and Eslick 2018).	**Satisfied**

8

Bradford Hill Analysis

Factor	Analysis	Weight
Biological Plausibility	Evidence shows that talcum powder ascends from the perineum through the vagina, cervix and uterus into the fallopian tubes and onto the ovary. Talcum powder is known to be an agent that causes inflammation. An inflammatory reaction caused by talcum powder on the tube and surface of the ovary results in genetic mutations and carcinogenesis. Talcum powder causes ovarian cancer through this mechanism. The "agent(s)" that causes the inflammatory reaction and carcinogenesis may be talc, asbestos, fibrous talc, heavy metals and/or chemicals contained in fragrances added to talcum powder.	**Critical Factor**
Coherence	Epidemiological data, in vitro and in vivo research are consistent in explaining the pathogenesis of EOC through the inflammatory mechanisms described above. Further, this is consistent with the causes of other cancers.	**Satisfied**
Experiment	There are no randomized trials comparing outcomes of women who use or who do not use talcum powder in their perineal hygiene. Further, such a trial at this point in time would be unethical. Laboratory research (in vitro) present evidence to support the biologic, genetic and epigenetic consequence to ovarian epithelium when exposed to talcum powder.	**Satisfied**
Analogy	There are numerous reports in the medical literature of minerals similar to talc causing cancer. Probably the most significant example is asbestos and lung cancer (mesothelioma).	**Satisfied**

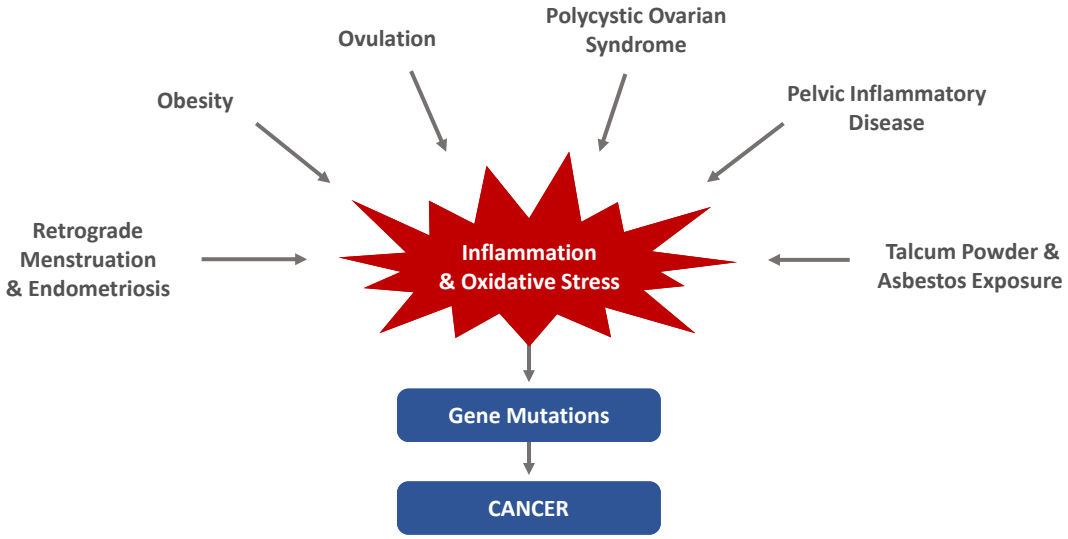
9

Risk Factors for Ovarian Cancer

- Inherited genetic mutations
  - BRCA1, BRCA2
- Family history of breast or ovarian cancer
- Age
- Lifetime ovulation
- Retrograde menstruation/endometriosis
- Talcum powder products
- Pelvic Inflammatory disease
- Obesity
- Polycystic ovarian disease
- Smoking

10

Inflammatory Risk Factors for Ovarian Cancer



11

Risk Factors

Hunn & Rodriguez (2012)

Ovarian Cancer: Etiology, Risk Factors, and Epidemiology

CLINICAL OBSTETRICS AND GYNECOLOGY
Volume 55, Number 1, 3–23
© 2012, Lippincott Williams & Wilkins

JESSICA HUNN, MD and GUSTAVO C. RODRIGUEZ, MD

(GC Opp. Ex. 124)

Mallen (2018)

Risk Factors for Ovarian Carcinoma

Adrianne R. Mallen, MD[a], Mary K. Townsend, ScD[b], Shelley S. Tworoger, PhD[b,c,*]

(GC Opp. Ex. 125)

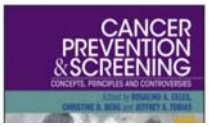
Vitonis (2011)

Assessing Ovarian Cancer Risk When Considering Elective Oophorectomy at the Time of Hysterectomy

Allison F. Vitonis, SM, Linda Titus-Ernstoff, PhD, and Daniel W. Cramer, MD, ScD
Obstet Gynecol. 2011 May ; 117(5): 1042–1050.

(PSC CP Ex. 1)

Gentry-Maharaj (2018)



CHAPTER 23
Ovarian cancer prevention and screening

(GC Opp. Ex. 127)

IOM Evolving Paradigms (2016)

Ovarian Cancers
EVOLVING PARADIGMS IN RESEARCH AND CARE
Committee on the State of the Science in Ovarian Cancer Research
Board on Health Care Services
Institute of Medicine

(GC Opp. Ex. 112)

Lheureux (2019)

Epithelial ovarian cancer

Stephanie Lheureux, Charlie Gourley, Ignace Vergote, Amit M Oza
Lancet 2019; 393: 1240-53

(GC Opp. Ex. 128)

12

LO4

Migration

Carbon Particles (1961)

The Transport of Carbon Particles in the Human Female Reproductive Tract

G. E. Egli, M.D., and Michael Newton, M.D.

(GC Opp. Ex. 67)

Radioactive Particles (1979)

2 June 1979 SA Medical Journal 917

Migration of a Particulate Radioactive Tracer from the Vagina to the Peritoneal Cavity and Ovaries

P. F. VENTER, M. ITURRALDE

(GC Opp. Ex. 70)

Retrograde Menstruation (1984)

Retrograde Menstruation in Healthy Women and in Patients With Endometriosis

JOUKO HALME, MD, PhD, MARY G. HAMMOND, MD, JAROSLAV F. HULKA, MD, SHAILAJA G. RAJ, MD, AND LUTHER M. TALBERT, MD

(GC Opp. Ex. 69)

Uterine Peristaltic Pump (1997)

THE UTERINE PERISTALTIC PUMP

Normal and Impeded Sperm Transport within the Female Genital Tract

G. Kunz,[1] D. Beil,[2] H. Deiniger,[2] A. Einspanier,[4] G. Mall,[3] and G. Leyendecker[1]

(GC Opp. Ex. 71)

Glove Powder (2004)

Retrograde migration of glove powder in the human female genital tract

A.C.E.Sjösten[1], H.Ellis[2] and G.A.B.Edelstam[1]

(GC Opp. Ex. 73)

Sperm (2006)

CHAPTER NINE

Gamete Transport and Fertilization

Jones & Lopez, "Human Reproductive Biology"

(GC Opp. Ex. 65)

CONFIDENTIAL - ATTORNEYS EYES ONLY

13

Talc and Asbestos in the Ovaries/Lymph Nodes

Talc in Ovaries (1971)

TALC AND CARCINOMA OF THE OVARY AND CERVIX

BY

W. J. Henderson, *Electron Microscopist*
Tenovus Institute for Cancer Research
C. A. F. Joslin, *Consultant Radiotherapist*
Velindre Memorial Centre for Cancer Research
A. C. Turnbull, *Professor of Obstetrics and Gynaecology*
Welsh National School of Medicine
AND
K. Griffiths, *Director*
Tenovus Institute for Cancer Research, Welsh National School of Medicine, Cardiff

BP Opp. Ex. 17

Talc in Ovaries (1996)

The relationship between perineal cosmetic talc usage and ovarian talc particle burden

Debra S. Heller, MD,* Carolyn Westhoff, MD,† Ronald E. Gordon, PhD,* and Norman Katz, AAS*
New York, New York

GC Opp. Ex. 75

Talc in Lymph Nodes (2007)

Presence of Talc in Pelvic Lymph Nodes of a Woman With Ovarian Cancer and Long-Term Genital Exposure to Cosmetic Talc

Daniel W. Cramer, MD, ScD, William R. Welch, MD, Ross S. Berkowitz, MD, and John J. Godleski, MD

GC Opp. Ex. 76

Asbestos in Ovaries (1996)

Asbestos Exposure and Ovarian Fiber Burden

Debra S. Heller, MD, Ronald E. Gordon, PhD, Carolyn Westhoff, MD, and Susan Gerber, MD

BP Opp. Ex. 49

14

Slide 13

LO4 Wendy, can you make sure the exhibit numbers on the lower right corner in the boxes show up completely? retrograde menstration is cut off

Leigh O'Dell, 7/21/2019

Regulatory Agents Agree Talcum Powder Migrates

Two theories have historically dominated on the cause of epithelial ovarian cancer and these are the "incessant ovulation hypothesis" and the "gonadotropin hypothesis." In addition to these endogenous factors, the role of exogenous factors via retrograde transport of noxious substances (e.g. carcinogens, particulates such as talc and asbestos, endometriosis and infectious agents) from the vagina and uterus into the Fallopian Tubes and peritoneal cavity have been studied extensively as a possible risk factor for ovarian cancer.

While there exists no direct proof of talc and ovarian carcinogenesis, the potential for particulates to migrate from the perineum and vagina to the peritoneal cavity is indisputable. It is, therefore, plausible that perineal talc (and other particulate) that

GC Opp. Ex. 78

15

Exposure to Asbestos and Fibrous Talc

1.6.5 Human exposure
(a) Exposure of the general population

Consumer products (e.g. cosmetics, pharmaceuticals) are the primary sources of exposure to talc for the general population. Inhalation and dermal contact (i.e. through perineal application of talcum powders) are the primary routes of exposure.

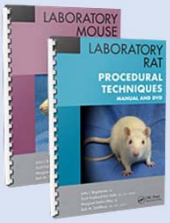
p. 232

GC Opp. Ex. 80

16

Talcum Powder Causes Inflammation

In Vitro – Cells



In Vivo – Animals

In Vivo – Humans

Inflammation

17

Health Canada – Indicative of Causal Effect

Health Canada

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. (P.iii)

GC Opp. Ex. 78

18

Exhibit 4

Arch Carson, MD, PhD

UTHealth
The University of Texas
Health Science Center at Houston
School of Public Health

- Associate Professor of Occupational Health, University of Texas Health Science Center, Houston
- Physician and scientist who specializes in the practice of medical toxicology
- Program Director NIOSH-funded Education and Research Center, University of Texas
- Program Director of Occupational and Environmental Medicine Residency Program, University of Texas, Health Science Center
- PhD, Kettering Laboratory, University of Cincinnati
- MD, The Ohio State University

Summary of Opinions

- Johnson's Baby Powder and Shower to Shower pose *a significant health hazard.*
- Epidemiological studies show a consistent positive relationship, amounting to about a 30% increased risk of ovarian cancer in genital talcum powder users.
- Talcum powder from personal hygiene applications to the perineum migrates through the reproductive tract onto the ovaries. Inhalation is a secondary route of exposure to the ovaries.
- Talcum powder produces chronic inflammation in the tissues in which it sequestered.
- Johnson's Baby Powder and Shower to Shower contains *mineral fibers* (asbestos and fibrous talc) that intensify the inflammatory response and stimulate cell growth and proliferation.
- Johnson's Baby Powder and Shower to Shower are carcinogenic.
- Regular genital use of Johnson's Baby Powder and Shower to Shower can cause epithelial ovarian cancer.

Methodology

STEP ONE: Risk Assessment

STEP TWO: Bradford Hill Considerations Analysis

Constituents in Johnson's Baby Powder & Shower to Shower

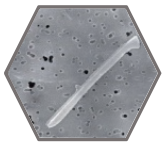
CONSTITUENTS IN BABY POWDER

The constituents shown below are found in Johnson's Baby Powder and Shower to Shower.

PLATY TALC

- Inflammatory
- Fibrogenic
- 2B carcinogen (IARC)

FIBROUS TALC



- Inflammatory; genotoxic
- Direct & indirect genotoxicity
- Group 1 carcinogen (IARC)

ASBESTOS

- Frequently present
- Direct & indirect genotoxicity
- All forms are Group 1 (IARC)

COBALT

Co

- Found in BP & S2S
- Oxidative stress
- 2B carcinogen (IARC)

CHROMIUM

Cr

- Found in BP & S2S
- Inflammation, OS, DNA damage
- Group 1 carcinogen (IARC)

NICKEL

Ni

- Found in BP & S2S
- Induces DNA damage
- Group 1 carcinogen (IARC)

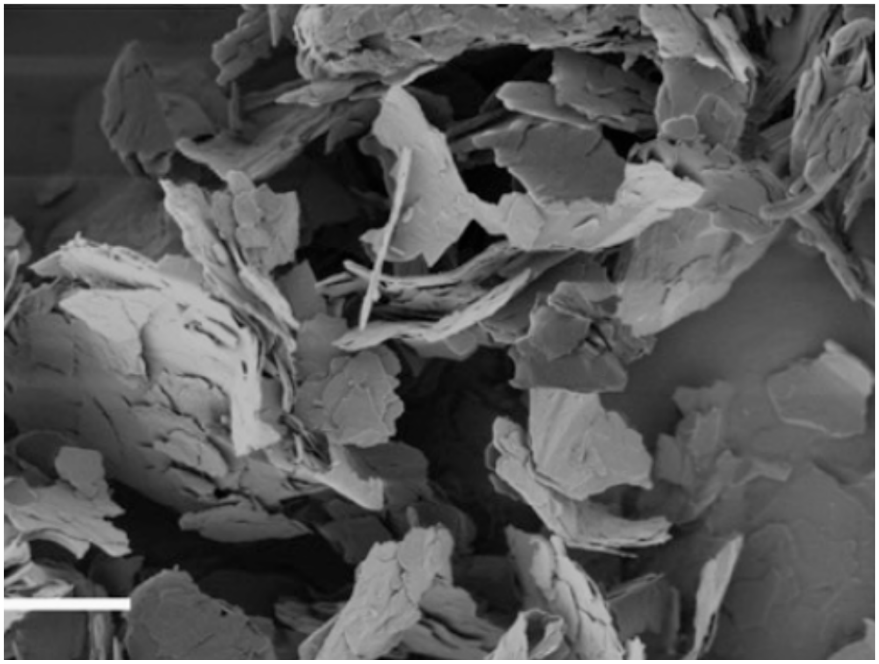
FRAGRANCE CHEMICALS

- Toxins & irritants
- Carcinogens – styrene (2A), and coumarin, eugenol, and d-limone (2B)

Fibrous talc and asbestos documented in Drs. Longo, Cook & Krekeler's Reports. (Asb. Opp. Exs. 67, 120, 119). Metals documented in Pier Ex. 47 and Drs. Cook and Krekeler's reports. Fragrance chemicals analyzed in Dr. Crowley's report. (HM Opp. Ex. 10).

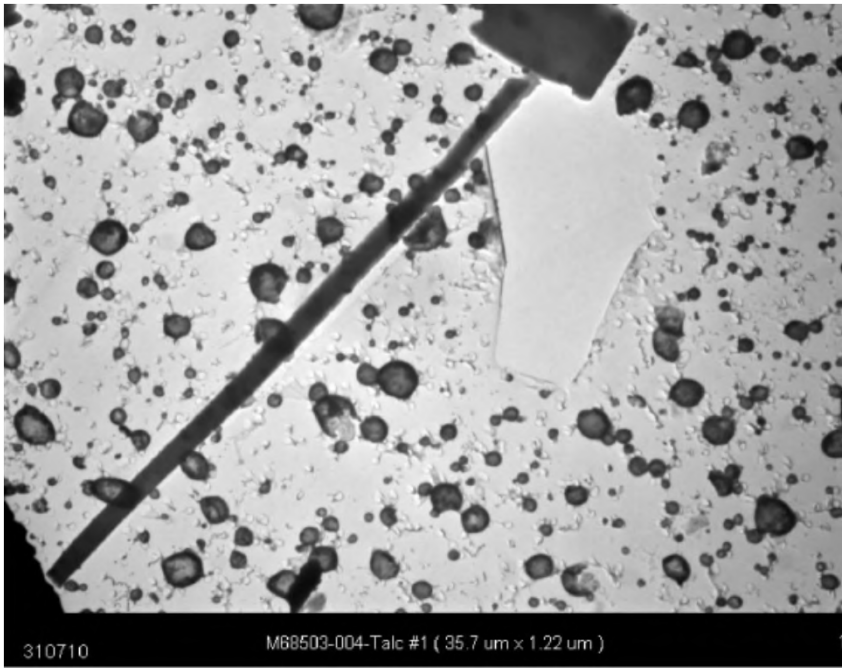
Platy Talc v. Fibrous Talc

Shukla (2009)



(GC Opp. Ex. 93)

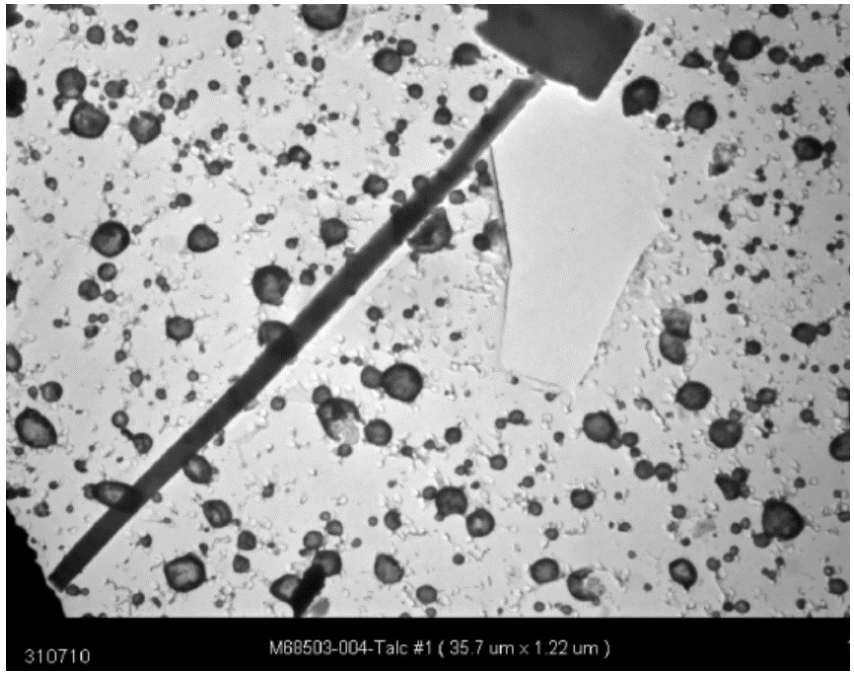
Longo Expert Report, p. 184



(Asbestos Opp. Ex. 67)

Fibrous Talc v. Asbestos

Longo Expert Report, p. 184



(Asbestos Opp. Ex. 67)

Longo Expert Report, p. 232

(Asbestos Opp. Ex. 67)

Asbestos in Platy Talc

Lockey (1981)

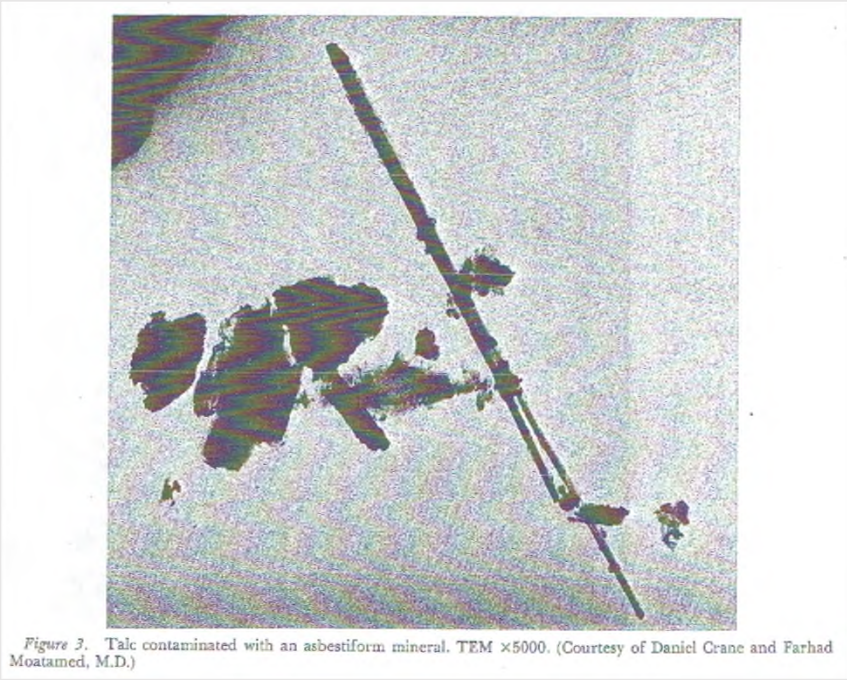
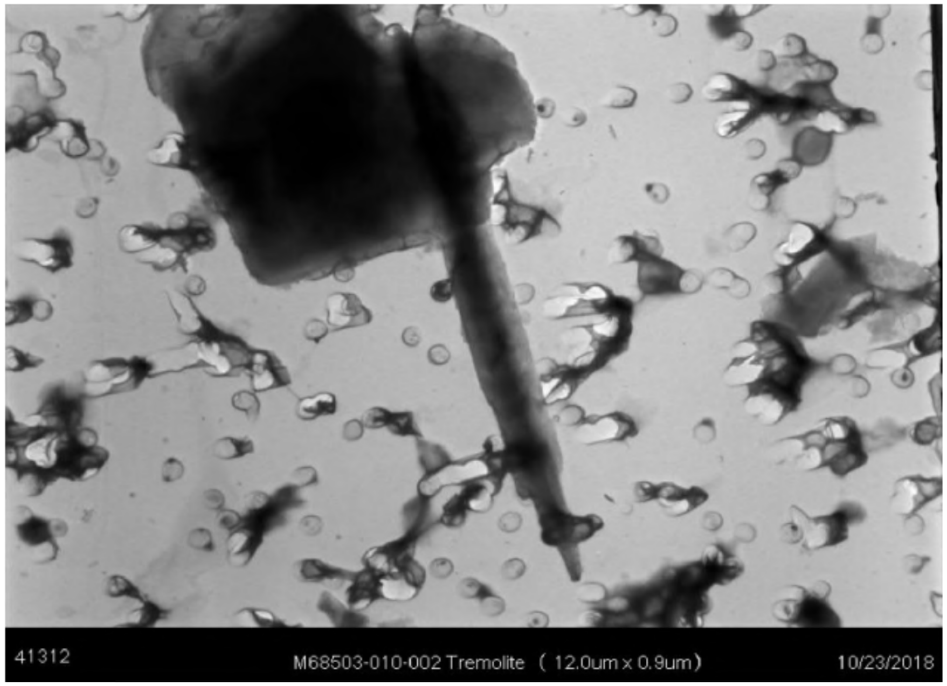


Figure 3. Talc contaminated with an asbestiform mineral. TEM ×5000. (Courtesy of Daniel Crane and Farhad Moatamed, M.D.)

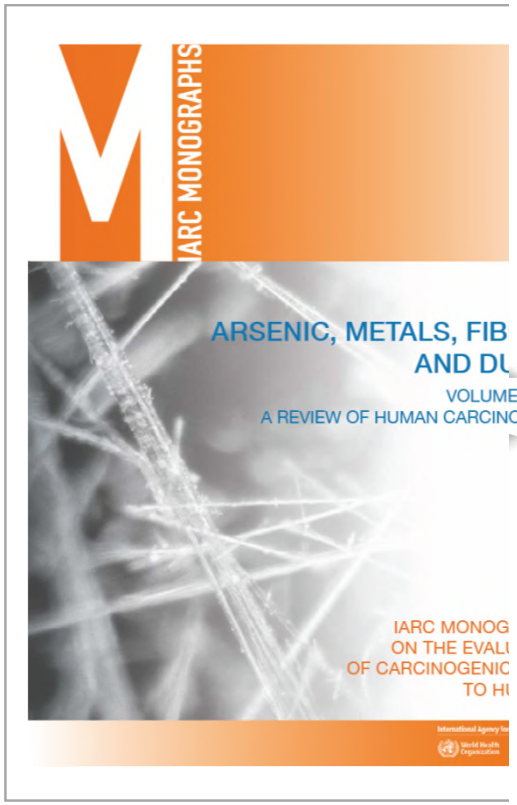
(Carson Ex. 10)

Longo Expert Report, p. 131



(Asbestos Opp. Ex. 67)

IARC – Fibrous Talc



IARC (2010)

""The review of talc in Supplement 7 led to evaluations for two agents: talc containing asbestiform fibres and talc not containing asbestiform fibres. The term 'asbestiform fibre' has been mistaken as a synonym for 'asbestos fibre' when it should be understood to mean any mineral, including talc, when it grows in an asbestiform habit." p.277

(GC Opp. Ex. 57)

IARC (2012)

"The conclusions reached in this Monograph about asbestos and its carcinogenic risks apply to these six types of fibres wherever they are found, and that includes talc containing asbestiform fibres." p. 219

"Talc may also form true mineral fibres that are asbestiform in habit." p. 230

(GC Opp. Ex. 80)

Constituents in Johnson's Baby Powder & Shower to Shower

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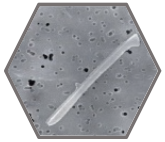

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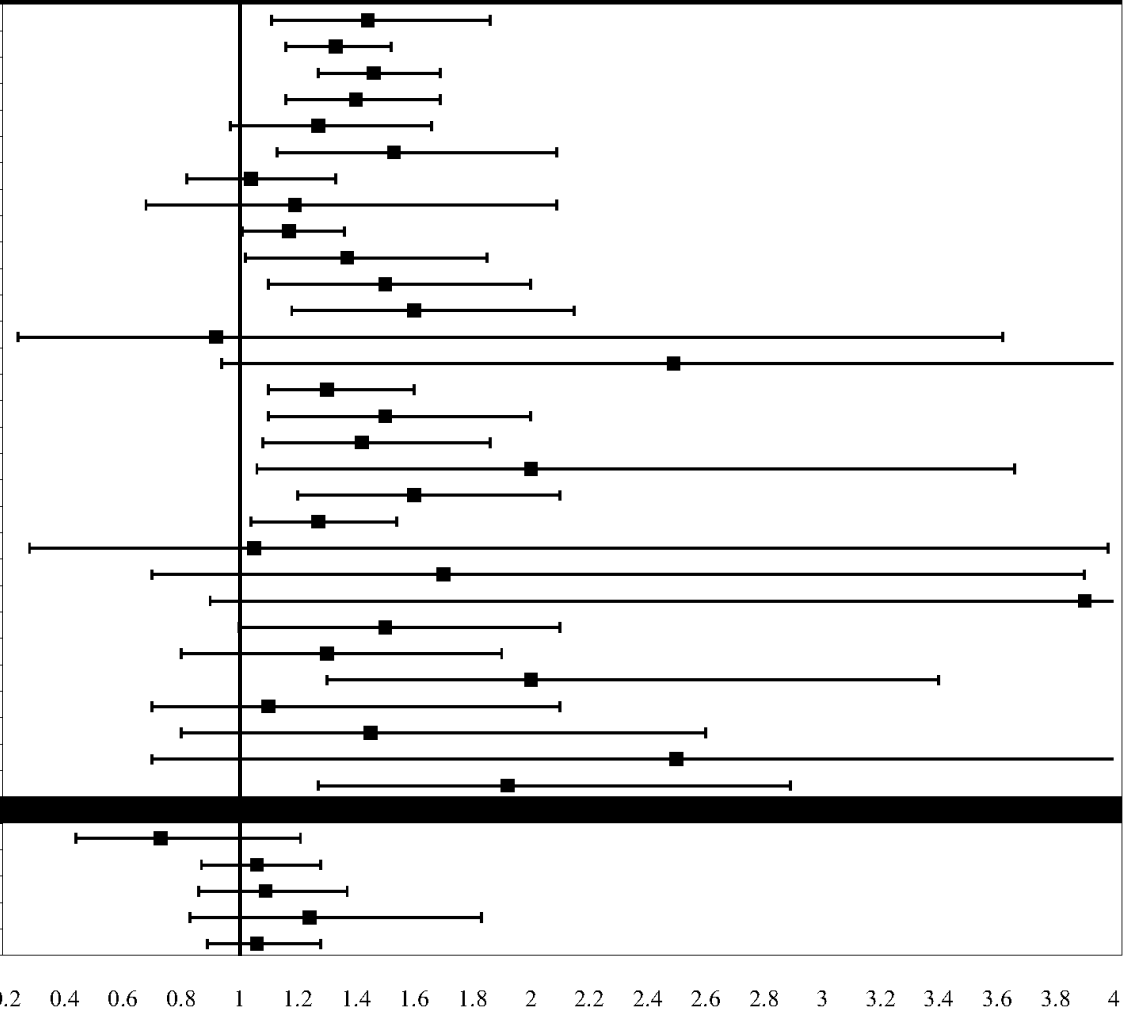
Case-Control and Cohort Studies (All Ovarian) (1982-2016)

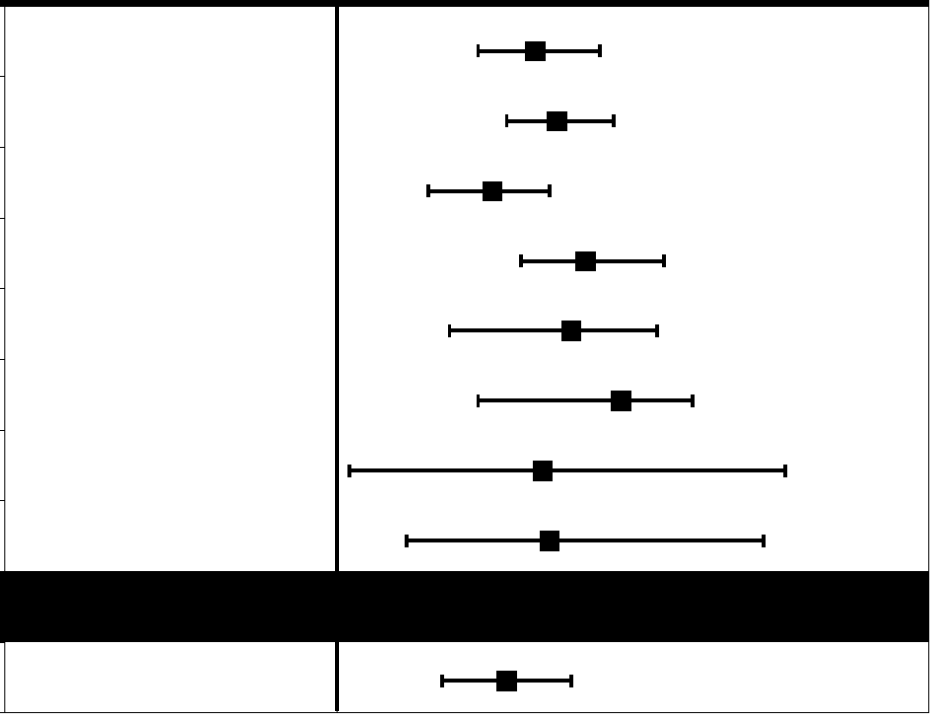
Case-Control Study	Natl	Cases	Ctrls	Pop	DR	RR	CI L	CI U
Schildkraut 2016	US	584	745	Pop.	Yes	1.44	1.11	1.86
Cramer 2016	US	2041	2100	Pop.	Yes	1.33	1.16	1.52
Wu 2015	US	1791	2391	Pop.	Yes	1.46	1.27	1.69
Kurta 2012	US	902	1802	Pop.	N/A	1.4	1.16	1.69
Rosenblatt 2011	US	812	1313	Pop.	No	1.27	0.97	1.66
Wu 2009	US	609	688	Pop.	Yes	1.53	1.13	2.09
Moorman 2009 (white)	US	1114	1086	Pop.	N/A	1.04	0.82	1.33
Moorman 2009 (black)	US	1114	1086	Pop.	N/A	1.19	0.68	2.09
Merritt 2008	Aus	1576	1509	Pop.	Yes	1.17	1.01	1.36
Mills 2004	US	256	1122	Pop.	No	1.37	1.02	1.85
Ness 2000	US	767	1367	Pop.	Incpl	1.5	1.1	2.0
Cramer 1999	US	563	523	Pop.	Yes	1.6	1.18	2.15
Wong 1999	US	499	755	Hosp.	Incpl	0.92	0.24	3.62
Godard 1998	Can	170	170	Pop.	N/A	2.49	0.94	6.58
Green 1997	Aus	824	855	Pop.	Incpl	1.3	1.1	1.6
Cook 1997	US	313	422	Pop.	No	1.5	1.1	2.0[1]
Chang 1997	Can	450	564	Pop.	No	1.42	1.08	1.86
Shushan 1996	Isr	200	408	Pop.	N/A	2	1.06[2]	3.66[2]
Cramer 1995	US	450	454	Pop.	N/A	1.6	1.2	2.1
Purdie 1995	Aus	824	860	Pop.	N/A	1.27	1.04	1.54
Tzonou 1993	Gre	189	200	Hosp.	N/A	1.05	0.28	3.98
Rosenblatt 1992	US	77	46	Hosp.	Yes	1.7	0.7	3.9
Chen 1992	Chn	112	224	Pop.	N/A	3.9	0.9	10.63
Harlow 1992	US	235	239	Pop.	Yes	1.5	1.0	2.1
Booth 1989 (daily)	UK	235	451	Hosp.	Yes	1.3	0.8	1.9[1]
Booth 1989 (weekly)	UK	235	451	Hosp.	Yes	2.0	1.3	3.4
Harlow 1989	US	116	158	Pop.	N/A	1.1	0.7	2.1
Whittemore 1988	US	188	539	H&P	Yes	1.45	0.81[1]	2.60[1]
Hartge 1983	US	135	171	Hosp.	N/A	2.5	0.7	10.0
Cramer 1982	US	215	215	Pop.	N/A	1.92	1.27	2.89
Cohort Studies	**Natl**	**Cases**	**Non**	**Flw Up**	**DR**	**RR**	**CI L**	**CI U**
Gonzalez (2016)	US	154	41,500	6.6	N/A	0.73	0.44	1.2[1]
Houghton (2014)	US	429	61,147	12.4	Incpl	1.06	0.87	1.28
Gertig (2000)	US	307	78,323	N/A	Incpl	1.09	0.86	1.37
Gates (2008)	US	210	600	N/A	Yes	1.24	0.83	1.83
Gates (2010)	US	797	78,323?	N/A	N/A	1.06	0.89	1.28



[1] Corrected data-point from study text (report figure: Cook 1997 CI Upper 2.3; Gonzalez CI Upper 1.21; Booth 1989 CI Upper 1.0; Whittemore CI p=0.06).
[2] Corrected data-point from defense expert report(s) (report figure: p=0.04).

Meta-Analyses and Pooled Studies (All Ovarian)

Study	#Studies	#Cases	DR	RR	CI L	CI U
Taher (2018)[1]	27	17,149	Yes	1.28	1.2	1.37
Penninkilampi (2018)	27	14,311	Yes	1.31	1.24	1.39
Berge (2018)[2]	27	N/A[3]	Yes	1.22	1.13	1.3
Langseth (2008)	20	N/A[3]	N/A	1.35	1.26	1.46
Huncharek (2003)	16	5260	No[4]	1.33	1.16	1.45
Cramer (1999)	14	3834	N/A	1.4	1.2	1.5
Gross (1995)	10[5]	1509	N/A	1.29	1.02	1.63
Harlow (1992)	6	1106	N/A	1.3	1.1	1.6
Pooled	**#Studies**	**#Cases**	**DR**	**RR**	**CI L**	**CI U**
Terry (2013)	8	8,525	Yes	1.24	1.15	1.33



[1] Taher was published after expert report completed.
[2] Updating to reflect 2018 version of same 2017 study cited in report.
[3] Number of cases not provided.
[4] No summary estimates calculated. Dose response addressed in 9/16 source studies: no dose-response apparent.
[5] N=5 studies with adjusted data and limited to epithelial ovarian cancers.

Migration

Carbon Particles (1961)

The Transport of Carbon Particles in the Human Female Reproductive Tract

G. E. Egli, M.D., and Michael Newton, M.D.

(GC Opp. Ex. 67)

Radioactive Particles (1979)

2 June 1979 SA MEDICAL JOURNAL 917

Migration of a Particulate Radioactive Tracer from the Vagina to the Peritoneal Cavity and Ovaries

P. F. VENTER, M. ITURRALDE

(GC Opp. Ex. 70)

Retrograde Menstruation (1984)

Retrograde Menstruation in Healthy Women and in Patients With Endometriosis

JOUKO HALME, MD, PhD, MARY G. HAMMOND, MD, JAROSLAV F. HULKA, MD, SHAILAJA G. RAJ, MD, AND LUTHER M. TALBERT, MD

(GC Opp. Ex. 69)

Uterine Peristaltic Pump (1997)

THE UTERINE PERISTALTIC PUMP

Normal and Impeded Sperm Transport within the Female Genital Tract

G. Kunz,[1] D. Beil,[2] H. Deiniger,[2] A. Einspanier,[4] G. Mall,[3] and G. Leyendecker[1]

(GC Opp. Ex. 69)

Glove Powder (2004)

Retrograde migration of glove powder in the human female genital tract

A.C.E.Sjösten[1], H.Ellis[2] and G.A.B.Edelstam[1]

(GC Opp. Ex. 73)

Talc Found In Tissue (1996)

The relationship between perineal cosmetic talc usage and ovarian talc particle burden

Debra S. Heller, MD,[a] Carolyn Westhoff, MD,[b] Ronald E. Gordon, PhD,[c] and Norman Katz. AAS[c]

New York, New York

(GC Opp. Ex. 75)

Regulatory Agencies Agree Talcum Powder Migrates

FDA

While there exists no direct proof of talc and ovarian carcinogenesis, the potential for particulates to migrate from the perineum and vagina to the peritoneal cavity is indisputable. It is, therefore, plausible that perineal talc (and other particulate) that reaches the endometrial cavity, Fallopian Tubes, ovaries and peritoneum may elicit a foreign body type reaction and inflammatory response that, in some exposed women, may progress to epithelial cancers. However, there has been no conclusive evidence to support causality.

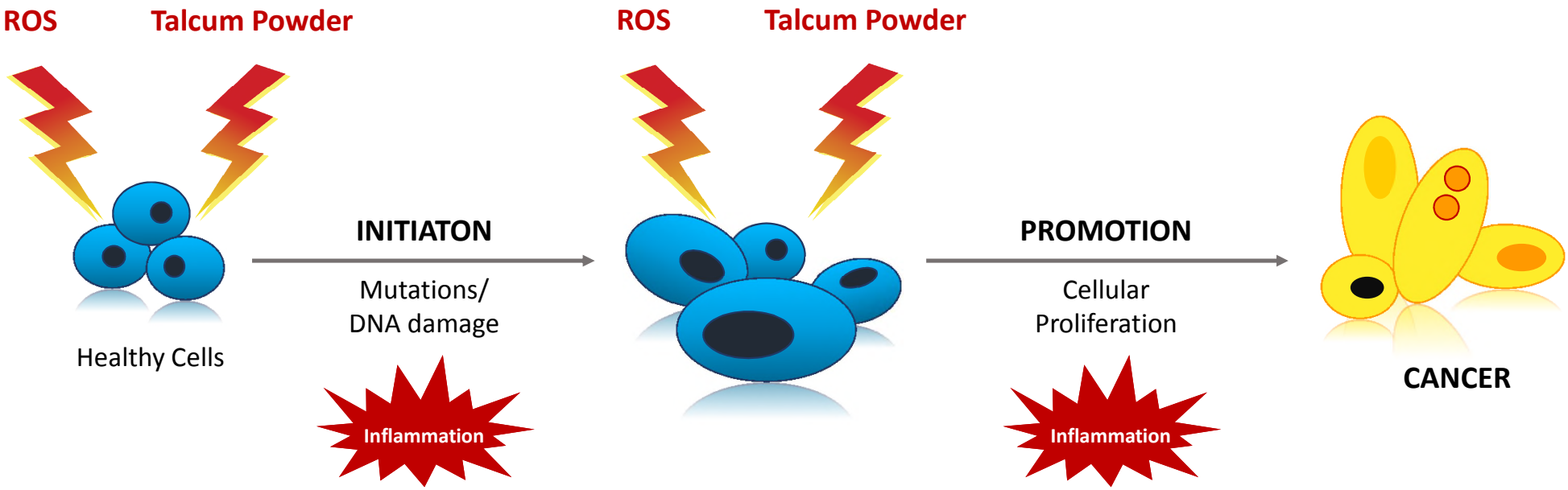
GC Opp. Ex. 78

Health Canada

Biological plausibility: Particles of talc are hypothesized to migrate into the pelvis and ovarian tissue, causing irritation and inflammation. The presence of talc in the ovaries has been documented (Heller et al. 1996b). This evidence of retrograde transport supports the biologic plausibility of the association between perineal talc application and ovarian exposure; however, the specific mechanism(s) and cascade of molecular events by which talc might cause ovarian cancer have not been identified (Taher et al. 2018).

GC Opp. Ex. 56

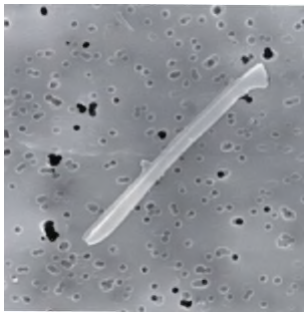
Carcinogenesis



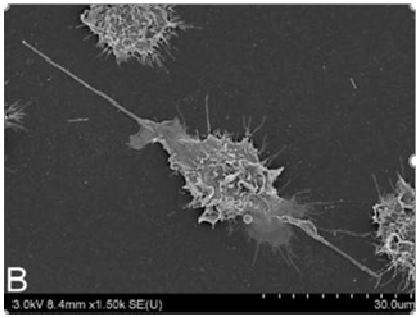
Cell Studies

Study	Cell Lines	Dose	Time	Findings
Shukla (2009) (GC Opp. Ex. 93)	• Immortalized ovarian mesothelial cells • Mesothelial cells	0, 1, 5, 10, 20, 75 µg/cm²	8 hrs. 24 hrs.	• Gene up-regulation
Buz'Zard (2007) (GC Opp. Ex. 94)	• Immortalized normal ovarian epithelial cells • Immortalized normal ovarian epithelial cells	0, 1, 5, 10, 15, 20, 50, 100, 500 µg/cm²	24 hrs. 72 hrs.	• Proliferation • Neoplastic transformation • Increased ROS
Akhtar (2010) (GC Opp. Ex. 96)	• Lung adenocarcinoma	1, 50, 100, 200 µg/mL	48 hrs.	• Oxidative stress
Akhtar (2014) (GC Opp. Ex. 95)	• Lung epithelial cells	0, 200 µg/mL	48 hrs.	• Cytotoxicity • Oxidative stress • Apoptosis
Fletcher, Saed (2019) (GC Opp. Ex. 97)	• Ovarian cancer cells A2780 • Ovarian cancer cells SKOV-3 • Ovarian cancer cells TOV112D • Normal macrophage cells • Primary normal ovarian epithelial cells • Immortalized ovarian epithelial cells • Immortalized fallopian tube cells	0, 5, 20, 100 µg/mL	72 hrs.	• Change in redox balance • Increased CA-125 • Increased cell proliferation • Decreased apoptosis • SNPs

The Impact of Fibers on Carcinogenesis



Fiber

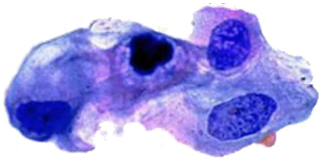


Inflammation

DIRECT

- DNA breaks & oxidized bases in DNA
- Fibers interfere with mitotic apparatus by direct physical interaction

Genotoxicity



Carcinogenesis

INDIRECT

- Oxidative stress
- Epigenetic alterations
- Actuation of signaling pathways
- Resistance to apoptosis
- Stimulation cell proliferation

Animal Studies

Eberl (1948)

"Incontrovertible evidence of the local irritant action of talcum"

(GC Opp. Ex. 81

Graham & Jenkins (1952)

"Talc was universally damaging"; in contrast, the starches seem to be relatively harmless

(GC Opp. Ex. 87)

Hamilton (1984)

Intrabursal injection of talc resulted in papillary transformation. Papillae may represent early neoplasia

(GC Opp. Ex. 90)

National Toxicology Program (1993)

Inhalation study with "non-fibrous talc." Toxic effects included chronic granulomatous inflammation, alveolar epithelial hyperplasia, squamous metaplasia and squamous cysts, and interstitial fibrosis of the lung.

(GC Opp. Ex. 88)

Keskin (2009)

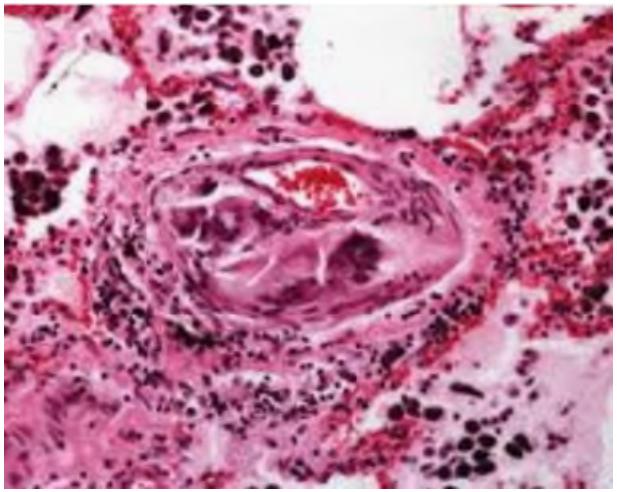
Talc has unfavorable effects on the female genital system (ovaries and fallopian tubes), causing tissue injury, macrophage infiltration, and an increased rate of infections and development of adhesions. (GC Opp. Ex. 91)

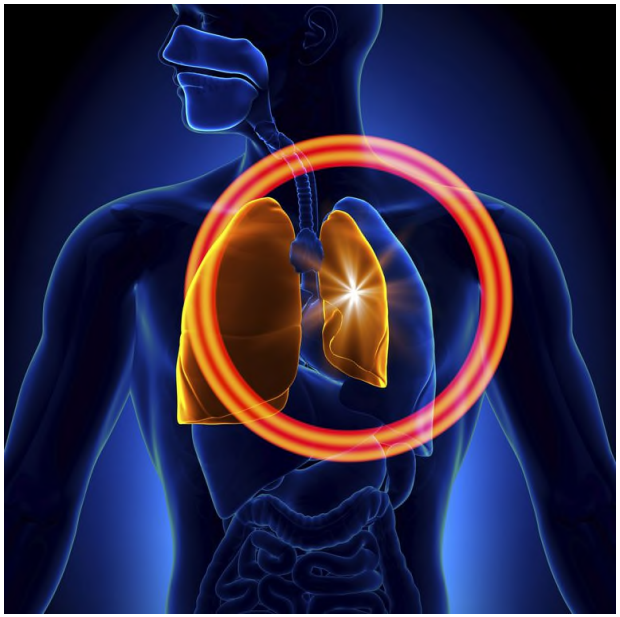
Moller & Jantzen (2013)

Meta-analysis of 64 publications on the association between exposure to asbestos particles and oxidatively damaged DNA in tissues of animals.

(GC Opp. Ex. 92)

Talc Produces Inflammation in Humans





Inflammation & Ovarian Cancer

Ness (1999)

The role of inflammation in ovarian epithelial cancer implicated talc and asbestos exposure.

(GC Opp. Ex. 106)

Reuter et al. (2010)

Ovarian cancer has been linked to reactive oxygen species.

(GC Opp. Ex. 103)

Balkwill & Mantovani (2001)

Recognizes talc is an inflammatory stimulus that is associated with ovarian cancer

(GC Opp. Ex. 66)

Trabert et al. (2014)

Multiple lines of evidence suggest that ovarian cancer may be related to chronic inflammation.

(GC Opp. Ex. 106)

Shan & Liu (2009)

Increasing evidence suggests inflammation contributes significantly to the etiology of epithelial ovarian cancer.

(GC Opp. Ex. 109)

Saed et al. (2017)

There is evidence supporting the role of oxidative stress in the etiology of ovarian cancer.

(GC Opp. Ex. 113)

Ness (1999)

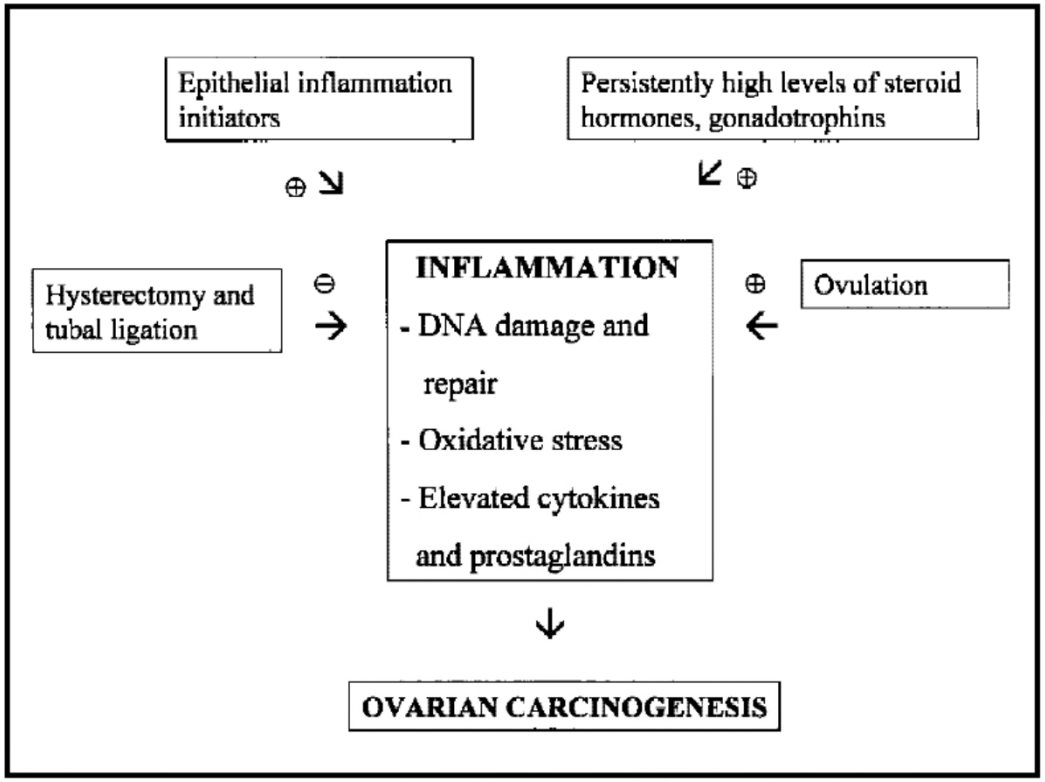
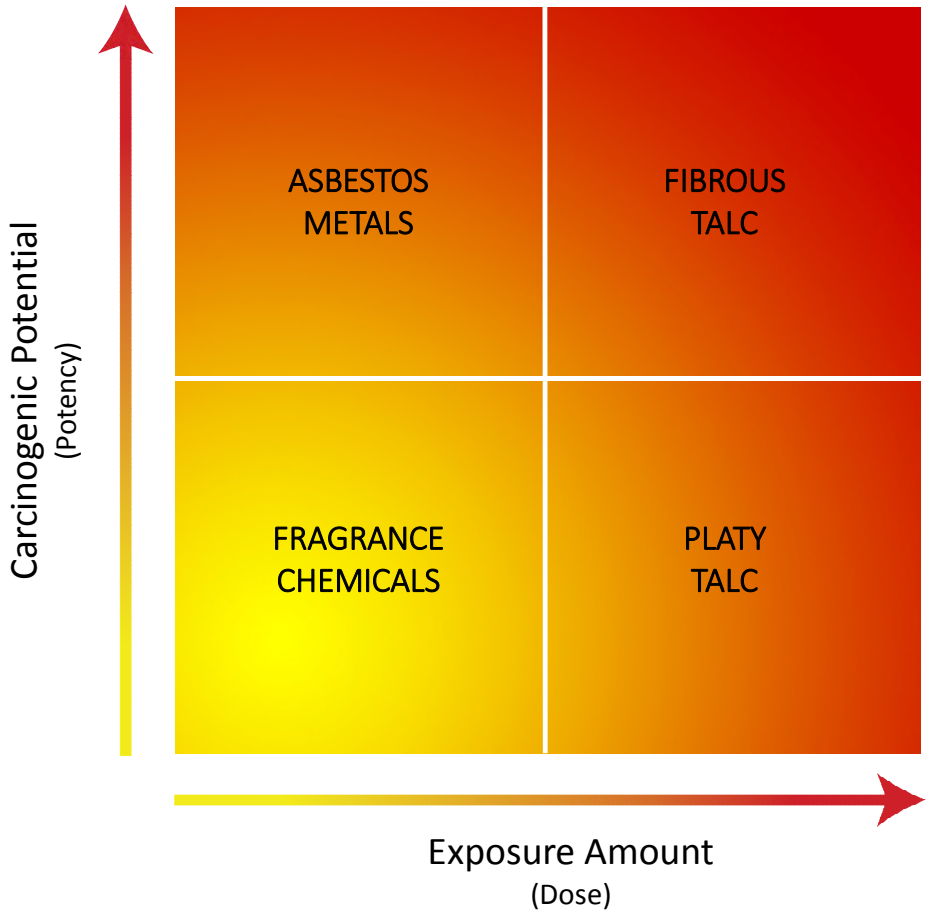


Fig. 1. Inflammation as a common mechanism underlying ovarian cancer.

(GC Opp. Ex. 106)

Baby powder contains multiple components that are carcinogenic



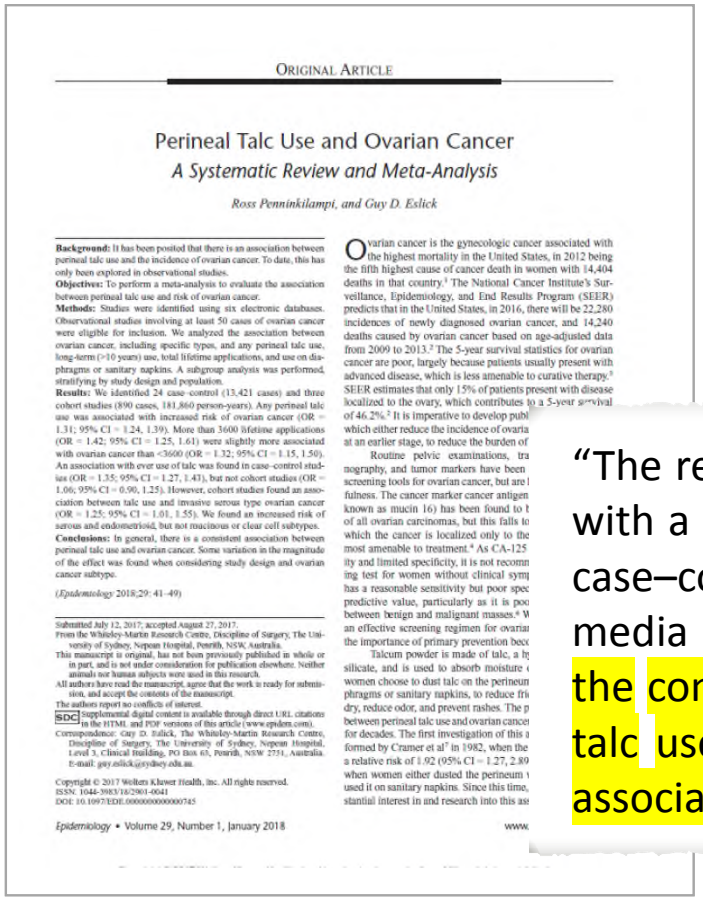
Bradford Hill Analysis

Factor	Analysis	Weight
Strength of Association	The majority case control studies (>25) showed positive and significant ORs for perineal talcum powder use and ovarian cancer. The three cohort studies did not find a significant relative risk but did show positive nonsignificant trends. Of the 8 meta-analyses, all showed statistically significant increased risk. These epidemiological studies support a strong association between the perineal use of talcum powder and ovarian cancer.	**Compelling**
Consistency	The majority of epidemiological studies that have investigated the link between perineal talcum powder use and ovarian cancer have reported positive associations. These studies are consistent in their findings of a relationship between perineal use of talcum powder products and the development of ovarian cancer. The meta-analyses have verified the comparability of the research methods used and the consensus of conclusions.	**Compelling**
Specificity	Although talcum powder is known to cause non-specific inflammation in many tissues where its residues locate, the stimulation of ovarian cancer is particularly associated with the presence of talc in the ovaries and fallopian tubes. Of known factors associated with ovarian cancer, i.e. nulliparous state, early menarche, late menopause, oral contraceptive use, living in the twentieth century and beyond, perineal talcum powder exposure is proving to be prominent among them.	**Satisfied**
Temporality	Studies investigating the link between perineal talcum powder exposure and ovarian cancer are designed to compare those with prior exposure to those who are not exposed, and so the scientific evidence supports this consideration.	**Satisfied**

Bradford Hill Analysis

Factor	Analysis	Weight
Biologic Gradient	Although some studies have failed to find evidence of a dose-response relationship, several more recent reports have shown a clear dose response when the number of subjects rose to a level producing sufficient statistical power to allow the analysis after subdivision of subjects into pertinent categorical groups, and frequency and duration were measured (Penninkilampi, 2018) (Schildkraut JM, 2016) (Cramer Daniel W, 2016) (Wu, et al., 2009).	**Satisfied**
Biologic Plausibility	Portions of the applied powders are transferred via active processes or passive mass action movements into the female reproductive tract, some making it all the way to the distal fallopian tubes, the ovary surfaces and the pelvic and peritoneal cavities. Once reaching the target tissues, talcum powder and its constituents initiate carcinogenesis via multiple means, including, inflammation with chemotaxis of inflammatory cells, liberation of cytokines, and reactive oxygen species, inactivation of TP53 genetic modulator, inhibition of DNA repair, and long-term promotion of genetic mutations via continuous inflammation and cellular growth stimulation.	**Compelling & well-described**
Coherence	The proposal that talcum powder product use results in the occurrence of ovarian cancer is entirely consistent with what is known about other factors related to ovarian cancer, i.e. early menarche, late menopause, pregnancies, breastfeeding history, oral contraceptive use, etc. All are factors that influence the local inflammatory environment of the ovary and its surroundings and have the potential to promote existing transcriptional errors and mutations.	**Satisfied**
Experiment	Interventions, such as tubal ligation that decreases the incidence of ovarian cancer by blocking the exposure route, offers experimental support for this mechanism. The use of cornstarch-based dusting powders as a substitute for talcum powder products offers additional experimental support.	**Satisfied**
Analogy	Asbestos is a mineral very similar both chemically and structurally to talc that has been found in the ovary and peritoneal cavity of exposed women. The mechanisms of carcinogenesis for both asbestos and talc are similar and analogous. Further, talc-based products contain asbestos and non-asbestos mineral fibers having carcinogenic potential.	**Satisfied**

Penninkilampi (2018)



ORIGINAL ARTICLE

Perineal Talc Use and Ovarian Cancer

A Systematic Review and Meta-Analysis

Ross Penninkilampi, and Guy D. Eslick

Background: It has been posited that there is an association between perineal talc use and the incidence of ovarian cancer. To date, this has only been explored in observational studies.

Objectives: To perform a meta-analysis to evaluate the association between perineal talc use and risk of ovarian cancer.

Methods: Studies were identified using six electronic databases. Observational studies involving at least 50 cases of ovarian cancer were eligible for inclusion. We analyzed the association between ovarian cancer, including specific types, and any perineal talc use, long-term (>10 years) use, total lifetime applications, and use on diaphragms or sanitary napkins. A subgroup analysis was performed, stratifying by study design and population.

Results: We identified 24 case–control (13,421 cases) and three cohort studies (890 cases, 181,860 person-years). Any perineal talc use was associated with increased risk of ovarian cancer (OR = 1.31; 95% CI = 1.24, 1.39). More than 3600 lifetime applications (OR = 1.42; 95% CI = 1.25, 1.61) were slightly more associated with ovarian cancer than <3600 (OR = 1.32; 95% CI = 1.15, 1.50). An association with ever use of talc was found in case–control studies (OR = 1.35; 95% CI = 1.27, 1.43), but not cohort studies (OR = 1.06; 95% CI = 0.90, 1.25). However, cohort studies found an association between talc use and invasive serous type ovarian cancer (OR = 1.25; 95% CI = 1.01, 1.55). We found an increased risk of serous and endometrioid, but not mucinous or clear cell subtypes.

Conclusions: In general, there is a consistent association between perineal talc use and ovarian cancer. Some variation in the magnitude of the effect was found when considering study design and ovarian cancer subtype.

(*Epidemiology* 2018;29: 41–49)

Ovarian cancer is the gynecologic cancer associated with the highest mortality in the United States, in 2012 being the fifth highest cause of cancer death in women with 14,404 deaths in that country.[1] The National Cancer Institute's Surveillance, Epidemiology, and End Results Program (SEER) predicts that in the United States, in 2016, there will be 22,280 incidences of newly diagnosed ovarian cancer, and 14,240 deaths caused by ovarian cancer based on age-adjusted data from 2009 to 2013.[2] The 5-year survival statistics for ovarian cancer are poor, largely because patients usually present with advanced disease, which is less amenable to curative therapy.[3] SEER estimates that only 15% of patients present with disease localized to the ovary, which contributes to a 5-year survival of 46.2%.[2] It is imperative to develop publ
which either reduce the incidence of ovaria
at an earlier stage, to reduce the burden of
Routine pelvic examinations, tra
nography, and tumor markers have been
screening tools for ovarian cancer, but are l
fulness. The cancer marker cancer antigen
known as mucin 16) has been found to b
of all ovarian carcinomas, but this falls to
which the cancer is localized only to the
most amenable to treatment.[4] As CA-125
ity and limited specificity, it is not recomm
ing test for women without clinical symp
has a reasonable sensitivity but poor spec
predictive value, particularly as it is poo
between benign and malignant masses.[6] W
an effective screening regimen for ovaria
the importance of primary prevention beco
Talcum powder is made of talc, a hy
silicate, and is used to absorb moisture
women choose to dust talc on the perineum
phragms or sanitary napkins, to reduce fri
dry, reduce odor, and prevent rashes. The p
between perineal talc use and ovarian cancer
for decades. The first investigation of this a
formed by Cramer et al[7] in 1982, when the
a relative risk of 1.92 (95% CI = 1.27, 2.89
when women either dusted the perineum
used it on sanitary napkins. Since this time,
stantial interest in and research into this as

Submitted July 12, 2017; accepted August 27, 2017.
From the Whiteley-Martin Research Centre, Discipline of Surgery, The University of Sydney, Nepean Hospital, Penrith, NSW, Australia.
This manuscript is original, has not been previously published in whole or in part, and is not under consideration for publication elsewhere. Neither animals nor human subjects were used in this research.
All authors have read the manuscript, agree that the work is ready for submission, and accept the contents of the manuscript.
The authors report no conflicts of interest.
SDC Supplemental digital content is available through direct URL citations in the HTML and PDF versions of this article (www.epidem.com).
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ORIGINAL ARTICLE

Perineal Talc Use and Ovarian Cancer

A Systematic Review and Meta-Analysis

Ross Penninkilampi, and Guy D. Eslick

"The results of this review indicate that perineal talc use is associated with a 24%–39% increased risk of ovarian cancer. While the results of case–control studies are prone to recall bias, especially with intense media attention following the commencement of litigation in 2014, the confirmation of an association in cohort studies between perineal talc use and serous invasive ovarian cancer is suggestive of a causal association."

GC Opp. Ex. 62)

Health Canada – Indicative of Causal Effect

Health Canada

The meta-analyses of the available human studies in the peer-reviewed literature indicate a consistent and statistically significant positive association between perineal exposure to talc and ovarian cancer. Further, available data are indicative of a causal effect. (P.iii)

(GC Opp. Ex. 56)

REDIRECT

Huncharek (2007)

422 Research paper

Use of cosmetic talc on contraceptive diaphragms a ovarian cancer: a meta-analysis of nine observation

Michael Huncharek[a,b], Joshua Muscat[c], Adedayo Onitilo[b] and Bruce Kupelnick[a]

Prior work suggests an association between perineal use of cosmetic talc and increased ovarian cancer risk. A meta-analysis was performed to examine this hypothesis by evaluating ovarian cancer risk associated with direct exposure of the female genital tract to talc via dusting of contraceptive diaphragms. Data were pooled from epidemiological studies using a general variance-based meta-analytic method that employs confidence intervals. The outcome of interest was a summary relative risk reflecting the risk of ovarian cancer development associated with the use of cosmetic talc on contraceptive diaphragms. Sensitivity analyses were performed to explain any observed statistical heterogeneity and to explore the influence of specific study characteristics on the summary estimate of effect. Initially, combining homogeneous data from nine case-control studies yielded a non-statistically significant summary relative risk of 1.03 (0.80–1.37), suggesting no association between talc-dusted diaphragms and ovarian cancer development. Sensitivity analyses were performed to evaluate the robustness of this finding. All resultant summary relative

risks were not statistically significant. epidemiological data do not support a between the use of cosmetic talc-dust ovarian cancer development. *Europea Prevention* 16:422–429 © 2007 Lippinc & Wilkins.

European Journal of Cancer Prevention 2007, 16

Keywords: diaphragms, ovarian neoplasms, systematic review, talcum powder

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Received 30 April 2006 Accepted 18 May 2006

Introduction

Ovarian cancer represents a major cause of cancer-related morbidity and mortality in the United States with an estimated 22 000 new cases diagnosed in 2005 (Boger-Megiddo and Weiss, 2005). It is the seventh most common cancer in women and ranks fourth as a cause of cancer deaths among female individuals from the United States, with some 16 000 succumbing to the disease this year. The lethality of ovarian tumors is in large part due to the fact that clinical symptoms tend to occur late in the natural history of the disease and the lack of screening tests allowing for early diagnosis. In fact, approximately 60% of patients are diagnosed with late-stage disease (stage III and IV) vastly diminishing the chance of long-term survival (approximately 10% at 5 years from diagnosis) (Richardson *et al.*, 1985).

Primary prevention of ovarian cancer remains elusive as a clear etiology for the vast majority of cases is unknown. Nonetheless, prior epidemiological research suggests a number of risk factors, including age (older versus younger), nulliparity, first pregnancy after the age of 35 years, diet high in saturated fats, positive family history of

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ovarian/breast cancer and race (wh American) (Baker and Piver, 1994; Mitchell, 1995; Daly and Obrams, graphic differences in incidence exist are found in industrialized countr developed nations (Ioka *et al.*, environmental factors in ovarian ca one exception is highly industrialize 2003) with a low annual incidence 3/100 000. Interestingly, Japanese woman who migrate to the United States experience an increased occurrence of this disease, further suggesting environmental factors in its cause.

In 1982, Cramer *et al.* (1982) published the first study suggesting a link between use of cosmetic talc and the risk of developing ovarian cancer. Subsequently, a number of additional reports have shown a small but increased risk among women using cosmetic talc products, although this finding is not universal (Chang and Risch, 1997). These statistical associations raise concerns that a cause–effect relationship may exist between talc exposure (particularly perineal use) and ovarian carcinogenesis.

Use of cosmetic talc on contraceptive diaphragms and risk of ovarian cancer: a meta-analysis of nine observational studies

Michael Huncharek[a,b], Joshua Muscat[c], Adedayo Onitilo[b] and Bruce Kupelnick[a]

"If one is exposed to a mixture of talc and asbestos, it is reasonable to expect a carcinogenic effect as it contains a known carcinogen." (p.427)

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GC Opp. Ex. 159)

Exhibit 5

The NEW ENGLAND JOURNAL of MEDICINE

EDITORIALS

New Guidelines for Statistical Reporting in the *Journal*

David Harrington, Ph.D., Ralph B. D'Agostino, Sr., Ph.D., Constantine Gatsonis, Ph.D., Joseph W. Hogan, Sc.D., David J. Hunter, M.B., B.S., M.P.H., Sc.D., Sharon-Lise T. Normand, Ph.D., Jeffrey M. Drazen, M.D., and Mary Beth Hamel, M.D., M.P.H

Some *Journal* readers may have noticed more parsimonious reporting of P values in our research articles over the past year. For example, in November 2018, we published two reports from the Vitamin D and Omega-3 Trial (VITAL),[1,2] a two-by-two factorial, placebo-controlled, randomized trial assessing whether vitamin D or marine n–3 (also known as omega-3) fatty acids prevent cardiovascular disease or cancer. For the n–3 portion of the trial, Manson et al.[2] reported 2 prespecified primary outcomes and 22 prespecified and other secondary outcomes — not uncommon in large, expensive randomized or observational studies. The n–3 fatty acids did not significantly reduce the rate of either the primary cardiovascular outcome or the cancer outcome. If reported as independent findings, the P values for two of the secondary outcomes would have been less than 0.05; however, the article reported only the hazard ratios and confidence intervals for the intervention effects for those secondary outcomes, consistent with recently implemented *Journal* guidelines limiting the use of P values for secondary and other comparisons.

We have now clarified, expanded, and refined our statistical guidelines for authors to cover both clinical trials and observational studies. The new guidelines discuss many aspects of the reporting of studies in the *Journal*, including a requirement to replace P values with estimates of effects or association and 95% confidence intervals when neither the protocol nor the statistical analysis plan has specified methods used to adjust for multiplicity. *Journal* editors and statistical consultants have become increasingly concerned about the overuse and misinterpretation of significance testing and P values in the medical literature. Along with their strengths, P values are subject to inherent weaknesses, as summarized in recent publications from the American Statistical Association.[3,4]

P values indicate how incompatible the observed data may be with a null hypothesis; "P<0.05" implies that a treatment effect or exposure association larger than that observed would occur less than 5% of the time under a null hypothesis of no effect or association and assuming no confounding. Concluding that the null hypothesis is false when in fact it is true (a type I error in statistical terms) has a likelihood of less than 5%. When P values are reported for multiple outcomes without adjustment for multiplicity, the probability of declaring a treatment difference when none exists can be much higher than 5%. When 10 tests are conducted, the probability that at least one of the 10 will have a P value less than 0.05 may be as high as 40% when the null hypothesis of no difference is true. Even when no adjustment for multiplicity is needed, P values do not represent the probability that the null hypothesis is false: P<0.05 does not imply that the probability of the null hypothesis is less than 5%. Because P values provide no information about the variability of an estimated association (its standard error), nonsignificant P values do not distinguish between group differences that are truly negligible and group differences that are noninformative because of large standard errors. P values provide no information about the size of an effect or an association.

The use of P values to summarize evidence in a study requires, on the one hand, thresholds that have a strong theoretical and empirical justification and, on the other hand, proper attention to the error that can result from uncritical interpretation of multiple inferences.[5] This inflation due to multiple comparisons can also occur when comparisons have been conducted by investigators but are not reported in a manuscript. A large array of methods to adjust for multiple comparisons is available and can be used to control the type I error probability in an analysis when specified in the design of a study.[6,7] Finally, the

notion that a treatment is effective for a particular outcome if P<0.05 and ineffective if that threshold is not reached is a reductionist view of medicine that does not always reflect reality.

Despite the difficulties they pose, P values continue to have an important role in medical research, and we do not believe that P values and significance tests should be eliminated altogether. A well-designed randomized or observational study will have a primary hypothesis and a prespecified method of analysis, and the significance level from that analysis is a reliable indicator of the extent to which the observed data contradict a null hypothesis of no association between an intervention or an exposure and a response. Clinicians and regulatory agencies must make decisions about which treatment to use or to allow to be marketed, and P values interpreted by reliably calculated thresholds subjected to appropriate adjustments have a role in those decisions.

The *Journal*'s revised policies on P values rest on three premises: it is important to adhere to a prespecified analysis plan if one exists; the use of statistical thresholds for claiming an effect or association should be limited to analyses for which the analysis plan outlined a method for controlling type I error; and the evidence about the benefits and harms of a treatment or exposure should include both point estimates and their margins of error.

We acknowledge that our new guidelines may present challenges in their use and interpretation, especially for authors and readers who are accustomed to thinking of P values or confidence intervals as a bright-line marker for a conclusion or a claim. We also understand that the results reported in a manuscript submitted to the *Journal* today may have come from a trial designed a decade ago. We are willing to work with authors within our new guidelines to maintain appropriate reporting of results. Finally, the current guidelines are limited to studies with a traditional frequentist design and analysis, since that matches the large majority of manuscripts submitted to the *Journal*. We do not mean to imply that these are the only acceptable designs and analyses. The *Journal* has published many studies with Bayesian designs and analyses[8-10] and expects to see more such trials in the future. When appropriate, our guidelines will be expanded to include best practices for reporting trials with Bayesian and other designs.

Disclosure forms provided by the authors are available with the full text of this editorial at NEJM.org.

From the Department of Data Sciences, Dana–Farber Cancer Institute (D.H.), Boston University (R.B.D.), Harvard T.H. Chan School of Public Health (D.H., D.J.H.), and the Department of Health Care Policy, Harvard Medical School, and the Department of Biostatistics, Harvard T.H. Chan School of Public Health (S.-L.T.N.) — all in Boston; the Department of Biostatistics and Center for Statistical Sciences, Brown University School of Public Health, Providence, RI (C.G., J.W.H.); and Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom (D.J.H.).

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HIV-1 Epidemic Control — Insights from Test-and-Treat Trials

Salim S. Abdool Karim, M.B., Ch.B., Ph.D.

Debate ensued when mathematical models[1] predicted that universal testing and treatment could achieve epidemic control within a few years in a high-burden setting. However, the idea rapidly gained new credence when antiretroviral therapy (ART)–induced viral suppression was shown to be highly effective in preventing transmission of human immunodeficiency virus type 1 (HIV-1) in a clinical trial.[2]

Four large, cluster-randomized, controlled trials, each with a different approach to maximizing viral suppression, set out to assess the effect of universal testing and treatment on community HIV incidence. In 2018, the Treatment as Prevention (TasP) trial[3] involving 28,419 persons in 22 communities showed no effect on HIV transmission in a rural South African district in which earlier

Exhibit 6

Research Article

Cancer Epidemiology, Biomarkers & Prevention

Douching, Talc Use, and Risk for Ovarian Cancer and Conditions Related to Genital Tract Inflammation

Iwona M. Gabriel[1], Allison F. Vitonis[1], William R. Welch[2], Linda Titus[3], and Daniel W. Cramer[1]

Abstract

Background: Douching is associated with disorders involving genital tract inflammation and genital talc use with epithelial ovarian cancer (EOC) but their joint effects are infrequently considered.

Methods: From 2,040 cases of EOC and 2,100 controls enrolled in Eastern Massachusetts and New Hampshire, we used unconditional logistic regression to estimate risk for EOC associated with douching and/or talc use. In subsets of cases and controls, we also collected information about pelvic inflammatory disease (PID), ectopic pregnancy, and cervical neoplasia to estimate risk for these events from douching and/or talc use.

Results: The adjusted OR and 95% confidence interval (CI) for all EOC was 0.94 (0.76–1.16) in women who douched but never used talc and 1.28 (1.09–1.51) in women who used talc but never douched. Compared with women who never regularly douched or used talc, ORs (95% CIs) were 0.83 (0.52–1.33) for women who both used talc and homemade douches and 1.53 (1.11–2.10) for women who both used talc and store-bought douches. Cases who both douched and used talc were more likely to have had PID compared with cases who had used neither, OR = 5.03 (95% CI, 1.61–15.7).

Conclusions: Douching is not an independent risk factor for ovarian cancer but the combination of talc use and store-bought douches may modestly increase the risk for EOC beyond that for talc use alone.

Impact: The joint effect of talc use and douching, especially with commercial products, should be considered in evaluating risks associated with disorders involving genital tract inflammation or EOC.

Introduction

Two relatively common feminine hygienic practices include vaginal douching and use of talc powders or sprays in the genital area. From a National Survey of U.S. women of reproductive age conducted in the late 1980s (1), 37% reported regular douching. A nearly identical proportion reported using talc in their genital area from a survey of older women conducted in the Northwest around the same time period (2). Reasons reported by women who douche include the desire for cleanliness and fresh smell (3), with use often around the time of menses or sexual activity. Because women who douche are also more likely to use talc, the latter group may have similar motivations (2). Epidemiologic factors associated with both practices include Black ethnicity, high body mass index (BMI), married status, and smoking (2, 3).

That a substantial proportion of women in the United States douche or use talc suggests these practices are widely perceived to be innocuous. However, epidemiologic studies suggest both may adversely affect reproductive health. Douching has been associated with pelvic inflammatory disease, ectopic pregnancy, cervical neoplasia, and bacterial and fungal vaginosis (4–9), and genital use of talc has been associated with increased risk of ovarian cancer (10). A recent study suggested that douching may also be associated with ovarian cancer (11); but whether talc use is associated with other adverse reproductive events, like pelvic inflammatory disease (PID) or cervical neoplasia linked to douching, has not been systematically investigated. A key issue in these studies is to what extent the factors that predispose women to douche or use talc use may also be independent risk factors for ovarian cancer or other adverse reproductive events, that is, how well has confounding been controlled for in the studies?

Here, we use data from a large case–control study of ovarian cancer conducted in New England between 1992 and 2008 with uniform data collected on talc use and douching. We estimated risk for ovarian cancer and other adverse reproductive outcomes associated with douching or genital talc use taking into consideration those factors that may influence why women choose to douche or use talc genitally.

Material and Methods

Data came from the three enrollment phases of the New England–based Case Control Study (phase I 1992–1997; phase II: 1998–2002; and phase III: 2003–2008). Details regarding enrollment are described elsewhere (10). Briefly, 3,957 women residing in Eastern Massachusetts and New Hampshire diagnosed with ovarian cancer between ages 18 and 80 were identified through tumor boards and registries. A total of 874 cases had either died or were ineligible because they had moved outside the study area, did not have a working telephone number, or had a

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non-ovarian primary tumor. Of the remaining 3,083 cases, 2,203 (71%) were enrolled. After excluding 128 non-epithelial and 35 mixed mesodermal tumors, 2,040 cases with epithelial tumors of ovarian, primary peritoneal, and fallopian tube origin, including borderline malignancies [henceforth, epithelial ovarian cancer (EOC)] were available for analysis.

Controls were identified through random digit dialing, driver license lists, and town-resident lists. Between 1992 and 1997, 420 (72%) women identified through random digit dialing and 102 (51%) women identified through lists agreed to participate. From 1998 to 2008, 4,366 potential controls were identified using the lists, of whom 1,426 (33%) were ineligible because they had died, moved, were too ill to participate, did not have a working phone, did not speak English, or had surgical removal of ovaries. Of eligible controls, 1,362 (46%) declined to participate by phone or via "opt-out" postcard and 1,578 (54%) were enrolled (2,100 total). Controls were frequency matched to cases by 5-year age groups and region of residence.

Exposure and outcome assessment

Subjects were interviewed in-person about potential EOC risk factors that occurred more than 1 year before diagnosis for cases and 1 year before date of interview for controls. Subjects were asked whether they "regularly" or "at least monthly" applied powder to: the genital or rectal area, sanitary napkins or tampons, underwear, or areas other than the genital–rectal area. Additional details included type of powder, age begun, years used, and applications per month. Lifetime exposure was estimated by multiplying the frequency of applications per month by months used. This was divided by 360 (i.e., daily use coded as 30/month) to yield talc-years. To create categorical variables for talc-years, we chose cut-off points based on quartiles for exposed controls and rounded to the nearest integer. Participants were asked whether they ever douched "regularly" and if they did, they were asked to provide the brand name or type of douches used, the age they began using them, and the total years used. We classified type of douche into any use of store-bought douche or homemade douches only. Women who used both store-bought and homemade douches (14 cases and seven controls) and women who said they used deodorant vaginal suppositories (two cases and two controls) were counted with those who used store-bought douches. In addition, we classified age at first use into three categories, <20, 20–29, and ≥30, and years of use into quartiles based on the control distribution of use.

Subjects were also asked about the occurrence of PID, ectopic pregnancy, and cervical neoplasia, the latter based upon either a history of cervical cancer or abnormal pap smear that required hysterectomy, conization, or a loop electrosurgical excision procedure. PID was assessed only in the last phase of the study, and cervical procedures were recorded only for study phases II–III. Risks for PID, ectopic pregnancy, and cervical neoplasia associated with talc use or douching were examined individually in EOC cases and controls separately.

Pathology reports were collected for all cases and reviewed by a gynecologic pathologist (W.R. Welch). Tumors were classified by behavior and histology (serous borderline, serous invasive, mucinous, endometrioid, and clear cell, other). Undifferentiated and transitional cell carcinomas, fallopian tube primaries, and primary peritoneal tumors were counted as serous. Mixed epithelial, malignant Brenner, and unspecified epithelial tumors were classified as other.

Statistical analysis

χ^2 tests were used to compare characteristics of cases and controls who did or did not douche or use talc in the genital area. We used unconditional logistic regression to estimate ORs and 95% confidence intervals (CI) for EOC. We examined the association between douching and EOC, stratified by genital talc use and the association between talc use and EOC, stratified by douching. We also examined these associations within histologic types of EOC. In addition, we modeled risk of adverse reproductive outcomes (PID, ectopic pregnancy, and cervical neoplasia) separately among cases and controls. Models were adjusted for the study matching factors (age, study center, and phase) and potential confounders including parity (continuous), oral contraceptive use (never, <23 months, 23–49 months, 50–96 months, and >96 months), BMI (continuous), race (white and non-white), diaphragm use (never and ever), spermicide use (never and ever), menopausal status (pre and post), marital status (never and ever married), smoking (never, former, and current), days of menstrual flow (≤5 and >5) and age at menarche (continuous), and tubal sterilization (yes and no). Tests for trend for duration of douching and talc-years were based on the Wald statistic using continuous variables weighted by category midpoints with zero assigned as the exposure for nonusers. Likelihood ratio tests comparing models with and without interaction terms were used to test for effect modification. Because exposure data were censored by date of diagnosis of ovarian cancer and not on the date of the adverse events, dose-response and trend analyses were not performed for those outcomes. Records with missing data for the exposure of interest were excluded from logistic regression models. Among model covariates, data were missing for BMI ($n = 11$), age at menarche ($n = 16$), and race ($n = 2$). Missing data points were assigned to the most common or median value for each variable to allow records with missing data to be included in multivariable models. Analyses were performed using SAS v9.4 (SAS Institute).

Ethical approval

Institutional review boards approved the study. All participants provided written informed consent.

Results

Several factors associated with the likelihood of douching were also associated with likelihood of using talc genitally and were seen in both cases and controls. Women more likely to engage in both practices were: older, postmenopausal, heavier, and married (Table 1). Women who douched were more likely to be smokers, parous, and have had a tubal ligation and less likely to have used oral contraceptives. Cases who used a diaphragm or had a tubal ligation and controls who used spermicides were more likely to have used talc. Among cases, douching and talc use varied by age at menarche but without any apparent trend for the practices to be associated with an earlier or later menarche. Among controls, those who reported more than 5 days of flow were more likely to douche than women with fewer days of flow. Accordingly, in subsequent tables, we adjusted for these factors in looking at risk for EOC or adverse reproductive outcomes.

Overall risk of EOC was not elevated for women who douched, compared with those who did not, OR (95% CI) = 0.98 (0.83–1.17; Table 2). The ORs for douching in relation to EOC were similar among women who used genital talc, OR = 1.03 (95% CI,

Q6

Table 1. Characteristics of cases and controls by douching and genital talc use

	Douching						Genital talc use					
	Cases			Controls			Cases			Controls		
	No	Yes	*P*	No	Yes	*P*	No	Yes	*P*	No	Yes	*P*
Age												
<50	699 (86.3%)	111 (13.7%)	0.001	744 (89.1%)	91 (10.9%)	<0.0001	599 (74.0%)	211 (26.0%)	<0.0001	670 (80.2%)	165 (19.8%)	<0.0001
50–64	683 (80.4%)	166 (19.6%)		705 (80.4%)	172 (19.6%)		541 (63.7%)	308 (36.3%)		599 (68.3%)	278 (31.7%)	
≥65	301 (79.0%)	80 (21.0%)		289 (74.5%)	99 (25.5%)		258 (67.7%)	123 (32.3%)		282 (72.7%)	106 (27.3%)	
Menopausal status												
Pre	776 (86.3%)	123 (13.7%)	<0.0001	830 (89.1%)	102 (10.9%)	<0.0001	652 (72.5%)	247 (27.5%)	0.0006	735 (78.9%)	197 (21.1%)	<0.0001
Post	907 (79.5%)	234 (20.5%)		908 (77.7%)	260 (22.3%)		746 (65.4%)	395 (34.6%)		816 (69.9%)	352 (30.1%)	
Center												
MA	1,343 (83.2%)	272 (16.8%)	0.13	1,424 (83.3%)	285 (16.7%)	0.15	1,082 (67.0%)	533 (33.0%)	0.004	1,232 (72.1%)	477 (27.9%)	0.0001
NH	340 (80.0%)	85 (20.0%)		314 (80.3%)	77 (19.7%)		316 (74.4%)	109 (25.6%)		319 (81.6%)	72 (18.4%)	
Study												
Phase I	460 (82.6%)	97 (17.4%)	0.97	419 (80.3%)	103 (19.7%)	0.13	408 (73.2%)	149 (26.8%)	0.01	430 (82.4%)	92 (17.6%)	<0.0001
Phase II	541 (82.2%)	117 (17.8%)		595 (82.5%)	126 (17.5%)		448 (68.1%)	210 (31.9%)		519 (72.0%)	202 (28.0%)	
Phase III	682 (82.7%)	143 (17.3%)		724 (84.5%)	133 (15.5%)		542 (65.7%)	283 (34.3%)		602 (70.2%)	255 (29.8%)	
Race												
White	1,627 (83.1%)	332 (16.9%)	0.005	1,710 (82.9%)	352 (17.1%)	0.13	1,343 (68.6%)	616 (31.4%)	0.97	1,526 (74.0%)	536 (26.0%)	0.25
Non-white[a]	56 (70.9%)	23 (29.1%)		28 (73.7%)	10 (26.3%)		54 (68.4%)	25 (31.6%)		25 (65.8%)	13 (34.2%)	
BMI[b]												
<20	141 (87.6%)	20 (12.4%)	<0.0001	123 (86.0%)	20 (14.0%)	0.0006	124 (77.0%)	37 (23.0%)	0.004	124 (86.7%)	19 (13.3%)	0.0005
20–24.9	745 (86.3%)	118 (13.7%)		794 (85.3%)	137 (14.7%)		608 (70.5%)	255 (29.5%)		692 (74.3%)	239 (25.7%)	
25–29.9	436 (78.4%)	120 (21.6%)		517 (82.3%)	111 (17.7%)		375 (67.4%)	181 (32.6%)		460 (73.2%)	168 (26.8%)	
≥30	359 (78.4%)	99 (21.6%)		296 (76.1%)	93 (23.9%)		289 (63.1%)	169 (36.9%)		267 (68.6%)	122 (31.4%)	
Smoking status												
Never	825 (86.0%)	134 (14.0%)	<0.0001	876 (87.0%)	131 (13.0%)	<0.0001	668 (69.7%)	291 (30.3%)	0.18	759 (75.4%)	248 (24.6%)	0.23
Former	598 (82.3%)	129 (17.7%)		635 (79.6%)	163 (20.4%)		480 (66.0%)	247 (34.0%)		573 (71.8%)	225 (28.2%)	
Current	260 (73.4%)	94 (26.6%)		227 (76.9%)	68 (23.1%)		250 (70.6%)	104 (29.4%)		219 (74.2%)	76 (25.8%)	
Married												
Never	303 (88.9%)	38 (11.1%)	0.0007	175 (90.7%)	18 (9.3%)	0.002	251 (73.6%)	90 (26.4%)	0.03	153 (79.3%)	40 (20.7%)	0.07
Ever	1,380 (81.2%)	319 (18.8%)		1,563 (82.0%)	344 (18.0%)		1,147 (67.5%)	552 (32.5%)		1,398 (73.3%)	509 (26.7%)	
Parity												
Nulliparous	572 (88.1%)	77 (11.9%)	<0.0001	335 (88.6%)	43 (11.4%)	0.0009	454 (70.0%)	195 (30.0%)	0.34	284 (75.1%)	94 (24.9%)	0.53
Parous	1,111 (79.9%)	280 (20.1%)		1,403 (81.5%)	319 (18.5%)		944 (67.9%)	447 (32.1%)		1,267 (73.6%)	455 (26.4%)	
OC use												
Never	786 (80.7%)	188 (19.3%)	0.04	612 (79.9%)	154 (20.1%)	0.008	672 (69.0%)	302 (31.0%)	0.67	559 (73.0%)	207 (27.0%)	0.49
Ever	897 (84.1%)	169 (15.9%)		1,126 (84.4%)	208 (15.6%)		726 (68.1%)	340 (31.9%)		992 (74.4%)	342 (25.6%)	
Tubal ligation												
No	1,471 (83.4%)	292 (16.6%)	0.005	1,406 (83.6%)	275 (16.4%)	0.03	1,222 (69.3%)	541 (30.7%)	0.05	1,241 (73.8%)	440 (26.2%)	0.95
Yes	212 (76.5%)	65 (23.5%)		332 (79.2%)	87 (20.8%)		176 (63.5%)	101 (36.5%)		310 (74.0%)	109 (26.0%)	
Diaphragm												
No	1,208 (82.1%)	264 (17.9%)	0.41	1,190 (82.1%)	259 (17.9%)	0.25	1,031 (70.0%)	441 (30.0%)	0.02	1,079 (74.5%)	370 (25.5%)	0.34
Yes	475 (83.6%)	93 (16.4%)		548 (84.2%)	103 (15.8%)		367 (64.6%)	201 (35.4%)		472 (72.5%)	179 (27.5%)	
Spermicides												
No	1,549 (82.8%)	322 (17.2%)	0.25	1,586 (82.7%)	332 (17.3%)	0.78	1,286 (68.7%)	585 (31.3%)	0.51	1,432 (74.7%)	486 (25.3%)	0.007
Yes	134 (79.3%)	35 (20.7%)		152 (83.5%)	30 (16.5%)		112 (66.3%)	57 (33.7%)		119 (65.4%)	63 (34.6%)	
Amount of flow												
Light/moderate	994 (82.9%)	205 (17.1%)	0.45	1,019 (83.7%)	199 (16.3%)	0.13	838 (69.9%)	361 (30.1%)	0.09	898 (73.7%)	320 (26.3%)	0.98
Moderate heavy/heavy	674 (81.6%)	152 (18.4%)		690 (81.1%)	161 (18.9%)		548 (66.3%)	278 (33.7%)		627 (73.7%)	224 (26.3%)	

(Continued on the following page)

Table 1. Characteristics of cases and controls by douching and genital talc use (Cont'd)

	Douching						Genital talc use					
	Cases			Controls			Cases			Controls		
	No	Yes	*P*	No	Yes	*P*	No	Yes	*P*	No	Yes	*P*
Age at menarche												
<12	347 (80.7%)	83 (19.3%)	0.01	344 (81.3%)	79 (18.7%)	0.65	286 (66.5%)	144 (33.5%)	0.02	311 (73.5%)	112 (26.5%)	0.93
12–13	976 (83.3%)	195 (16.7%)		980 (83.5%)	194 (16.5%)		786 (67.1%)	385 (32.9%)		872 (74.3%)	302 (25.7%)	
14	198 (86.8%)	30 (13.2%)		202 (83.5%)	40 (16.5%)		175 (76.8%)	53 (23.2%)		175 (72.3%)	67 (27.7%)	
>14	154 (75.9%)	49 (24.1%)		205 (81.0%)	48 (19.0%)		145 (71.4%)	58 (28.6%)		186 (73.5%)	67 (26.5%)	
Days of flow												
≤5	1,128 (82.5%)	239 (17.5%)	0.92	1,192 (83.8%)	230 (16.2%)	0.05	935 (68.4%)	432 (31.6%)	0.96	1,033 (72.6%)	389 (27.4%)	0.07
>5	541 (82.3%)	116 (17.7%)		534 (80.3%)	131 (19.7%)		450 (68.5%)	207 (31.5%)		508 (76.4%)	157 (23.6%)	

Abbreviation: OC, oral contraceptive.
[a]Non-white race includes 58 African American, 64 Hispanic, 46 Asian, and 9 other race women.
[b]BMI is missing for two cases and nine controls.

0.77–1.38) and those who did not, OR = 0.94 (95% CI, 0.76–1.16). Excluding women with tubal ligation (rather than adjusting for it) did not materially change these estimates; OR = 0.98 (95% CI, 0.81–1.19) for douching overall, OR = 1.09 (95% CI, 0.79–1.52) for douching and talc, and OR = 0.93 (95% CI, 0.73–1.18) for douching alone. No trends in overall risk for EOC were associated with age-at-first use of douching or years of douching overall or in subgroups of women who used or did not use talc. Risk of EOC overall appeared to be decreased with use of "homemade" douching products OR (95% CI), 0.78 (0.60–1.02) whereas risk was increased with use of "store-bought" products, OR = 1.11 (0.91–1.37), but neither association was statistically significant. This difference was more apparent among women who used talc but did not reach significance in tests for heterogeneity (see Table 2 footnote).

In Table 3, we show the findings for talc use overall and in analyses stratified by douching. Women who used talc had an elevated risk for EOC overall compared with those who did not, OR (95% CI), 1.30 (1.13–1.50). The ORs for talc use in relation to EOC were similar among women who had also regularly douched, OR, 1.32 (95% CI, 0.95–1.82) and those who had not, OR, 1.28 (95% CI, 1.09–1.51). Excluding women with tubal ligation slightly lowered these estimates but did not change their significance; OR, 1.23 (95% CI, 1.05–1.44) for talc use overall, OR, 1.33 (95% CI, 0.92–1.92) for talc and douching, and OR, 1.19 (95% CI, 1.00–1.42) for talc alone. Risks were greater for women who began talc use during their 20s, and this was true regardless of whether the woman also douched. Risk of EOC increased significantly with increasing talc-years and the trend was more apparent in women who did not regularly douche. The ORs associated with ever-use of talc, age-at-first use, and talc-years of use were not significantly different among women who had also douched and those who had not (see Table 3 footnote).

Table 4 examines risk for EOC overall and for specific histologic types of ovarian cancer in four mutually exclusive usage categories: women who never douched or used talc, women who used talc but did not douche, women who douched but did not use talc, and women who both douched and used talc. Douching, compared with neither douching nor using talc, did not increase risk for EOC overall or histologic subtypes, and this was true whether the douching product was store-bought or homemade.

Compared with not douching or using talc, the OR for using talc was elevated for EOC overall (OR, 1.29; 95% CI, 1.10–1.51), for serous borderline tumors (OR, 1.39; 95% CI, 0.99–1.97), and for serous invasive tumors (OR, 1.39; 95% CI, 1.14–1.69). The associations were slightly stronger for women who used talc and store-bought douches, compared with those who used neither; OR, 1.53 (95% CI, 1.11–2.10) for EOC overall, OR, 2.11 (95% CI, 1.13–3.96) for serous borderline, and OR, 1.57 (95% CI, 1.07–2.31) for serous invasive tumors. Risk for the endometrioid subtype was elevated for those who used talc and store-bought douches compared with talc use without douching, but the association was not statistically significant. Although these findings are suggestive of an interaction between talc use and store-bought douches, formal tests for interaction did not reach the level of statistical significance (see Table 4 footnote).

Table 5 shows the risk for PID, ectopic pregnancy, and cervical neoplasia in cases and controls separately, again by the mutually exclusive categories related to talc and type of douche used. Relative to cases who neither douched nor used talc, elevated risks for PID were found for cases who used a store-bought douche

Table 2. Associations between douching and ovarian cancer by talc use

Douching	Controls *N* (%)	Cases *N* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	*P*[a]
			All cases and controls		
Douched regularly					
No	1,738 (82.8)	1,683 (82.5)	1.00 (reference)	1.00 (reference)	
Yes	362 (17.2)	357 (17.5)	1.02 (0.87–1.20)	0.98 (0.83–1.17)	0.85
Age at first use					
<20	90 (4.3)	100 (4.9)	1.15 (0.86–1.54)	1.12 (0.82–1.52)	0.48
20–29	217 (10.4)	184 (9.0)	0.88 (0.71–1.08)	0.84 (0.68–1.05)	0.12
≥30	51 (2.4)	68 (3.3)	1.38 (0.95–1.99)	1.34 (0.91–1.97)	0.14
Duration of douching					
≤5 years	92 (4.4)	94 (4.6)	1.06 (0.79–1.42)	1.04 (0.76–1.41)	0.82
6–15 years	92 (4.4)	101 (5.0)	1.13 (0.85–1.52)	1.12 (0.83–1.52)	0.46
16–26 years	87 (4.2)	72 (3.5)	0.86 (0.62–1.18)	0.80 (0.57–1.11)	0.18
>26 years	87 (4.2)	84 (4.1)	1.00 (0.73–1.36)	0.93 (0.67–1.29)	0.68
P_{trend}					0.50
Type of douche used					
Store-bought	217 (10.3)	239 (11.7)	1.14 (0.94–1.38)	1.11 (0.91–1.37)	0.30
Homemade	142 (6.8)	114 (5.6)	0.83 (0.64–1.07)	0.78 (0.60–1.02)	0.07
			Among talc users		
Douched regularly[b]					
No	428 (78.0)	496 (77.3)	1.00 (reference)	1.00 (reference)	
Yes	121 (22.0)	146 (22.7)	1.04 (0.79–1.37)	1.03 (0.77–1.38)	0.84
Age at first use[c]					
<20	26 (4.8)	45 (7.1)	1.49 (0.91–2.46)	1.45 (0.85–2.46)	0.17
20–29	74 (13.5)	71 (11.1)	0.83 (0.58–1.18)	0.82 (0.57–1.19)	0.29
≥30	19 (3.5)	26 (4.1)	1.18 (0.64–2.16)	1.17 (0.62–2.20)	0.64
Duration of douching[d]					
≤5 years	27 (4.9)	33 (5.2)	1.05 (0.62–1.78)	1.12 (0.65–1.93)	0.68
6–15 years	32 (5.9)	43 (6.7)	1.16 (0.72–1.87)	1.18 (0.72–1.95)	0.51
16–26 years	30 (5.5)	29 (4.5)	0.83 (0.49–1.41)	0.69 (0.39–1.21)	0.20
>26 years	29 (5.3)	37 (5.8)	1.10 (0.67–1.82)	1.09 (0.64–1.87)	0.75
P_{trend}					0.91
Type of douche used[e]					
Store-bought	75 (13.7)	108 (16.9)	1.24 (0.90–1.71)	1.22 (0.87–1.71)	0.25
Homemade	45 (8.2)	35 (5.5)	0.67 (0.42–1.06)	0.67 (0.41–1.10)	0.11
			Among those who never used talc		
Douched regularly[b]					
No	1310 (84.5)	1187 (84.9)	1.00 (reference)	1.00 (reference)	
Yes	241 (15.5)	211 (15.1)	0.97 (0.79–1.18)	0.94 (0.76–1.16)	0.58
Age at first use[c]					
<20	64 (4.1)	55 (3.9)	0.95 (0.66–1.37)	0.94 (0.63–1.39)	0.74
20–29	143 (9.2)	113 (8.1)	0.87 (0.67–1.13)	0.84 (0.64–1.10)	0.20
≥30	32 (2.1)	42 (3.0)	1.45 (0.91–2.31)	1.48 (0.91–2.42)	0.11
Duration of douching[d]					
≤5 years	65 (4.2)	61 (4.4)	1.04 (0.72–1.48)	1.00 (0.69–1.44)	0.98
6–15 years	60 (3.9)	58 (4.2)	1.07 (0.74–1.54)	1.08 (0.74–1.59)	0.69
16–26 years	57 (3.7)	43 (3.1)	0.83 (0.56–1.25)	0.83 (0.55–1.27)	0.40
>26 years	58 (3.7)	47 (3.4)	0.89 (0.60–1.32)	0.81 (0.54–1.24)	0.33
P_{trend}					0.33
Type of douche used[e]					
Store-bought	142 (9.2)	131 (9.4)	1.02 (0.79–1.31)	1.01 (0.78–1.32)	0.92
Homemade	97 (6.3)	79 (5.7)	0.90 (0.66–1.22)	0.85 (0.61–1.18)	0.33

NOTE: The following variables have missing data: age at first use (n = 9), duration (n = 10), and type of douche (n = 7).

[a]Adjusted for age, study center and phase, menopausal status, marital status, parity, oral contraceptive use and duration, tubal ligation, BMI, race, diaphragm use, spermicide use, smoking, days of menstrual flow, and age at menarche.

[b]$P_{heterogeneity} = 0.79$.

[c]$P_{heterogeneity} = 0.43$.

[d]$P_{heterogeneity} = 0.91$.

[e]$P_{heterogeneity} = 0.50$.

alone OR, 4.44 (95% CI, 1.22–16.1) and for those who used a store-bought douche and talc, OR, 5.46 (95% CI, 1.64–18.2). An elevated risk for CIN in cases who used homemade douches was also seen. Risk estimates for these associations were imprecise as illustrated by their wide CIs. For controls, none of the ORs reached significance nor were differences in risk found by whether homemade or store-bought douches were used.

Discussion

Using data from a case–control study of ovarian cancer, we examined the role of douching as a risk factor for EOC independent of talc use and, conversely, whether talc use affects risks for adverse reproductive outcomes that have been associated with douching such as PID. Examined as separate variables, douching

Table 3. Associations between genital talc use and ovarian cancer by douching

Genital talc use	Controls *N* (%)	Cases *N* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	*P*[a]
			All cases and controls		
Ever used					
No	1551 (73.9)	1398 (68.5)	1.00 (reference)	1.00 (reference)	
Yes	549 (26.1)	642 (31.5)	1.30 (1.13–1.48)	1.30 (1.13–1.50)	0.0003
Age at first use					
<20	343 (16.4)	363 (17.9)	1.17 (1.00–1.38)	1.15 (0.97–1.37)	0.10
20–29	122 (5.8)	183 (9.0)	1.66 (1.31–2.12)	1.73 (1.35–2.23)	<0.0001
≥30	76 (3.6)	87 (4.3)	1.27 (0.93–1.74)	1.25 (0.90–1.74)	0.18
Talc-years					
≤1 talc-year	138 (6.6)	138 (6.8)	1.11 (0.87–1.42)	1.12 (0.86–1.45)	0.40
>1–5 talc-years	124 (5.9)	148 (7.3)	1.32 (1.03–1.70)	1.37 (1.05–1.77)	0.02
>5–24 talc-years	146 (7.0)	170 (8.4)	1.29 (1.02–1.63)	1.24 (0.97–1.58)	0.08
>26 talc-years	127 (6.1)	171 (8.4)	1.49 (1.17–1.90)	1.51 (1.17–1.95)	0.001
P_{trend}					0.0001
			Among women who douched		
Ever used[b]					
No	241 (66.6)	211 (59.1)	1.00 (reference)	1.00 (reference)	
Yes	121 (33.4)	146 (40.9)	1.38 (1.02–1.87)	1.32 (0.95–1.82)	0.10
Age at first use[c]					
<20	80 (22.2)	85 (23.9)	1.21 (0.85–1.73)	1.15 (0.78–1.69)	0.47
20–29	25 (6.9)	45 (12.6)	2.06 (1.22–3.47)	2.04 (1.17–3.55)	0.01
≥30	14 (3.9)	15 (4.2)	1.22 (0.58–2.59)	1.19 (0.54–2.62)	0.67
Talc-years[d]					
≤1 talc-year	24 (6.7)	26 (7.3)	1.24 (0.69–2.22)	1.31 (0.69–2.47)	0.41
>1–5 talc-years	19 (5.3)	30 (8.5)	1.80 (0.99–3.30)	1.89 (1.00–3.57)	0.05
>5–24 talc-years	40 (11.1)	40 (11.3)	1.14 (0.71–1.84)	0.95 (0.57–1.59)	0.85
>26 talc-years	36 (10.0)	47 (13.3)	1.49 (0.93–2.39)	1.47 (0.90–2.43)	0.13
P_{trend}					0.15
			Among women who did not douche		
Ever used[b]					
No	1310 (75.4)	1187 (70.5)	1.00 (reference)	1.00 (reference)	
Yes	428 (24.6)	496 (29.5)	1.28 (1.10–1.49)	1.28 (1.09–1.51)	0.002
Age at first use[c]					
<20	263 (15.2)	278 (16.6)	1.17 (0.97–1.41)	1.15 (0.94–1.39)	0.17
20–29	97 (5.6)	138 (8.2)	1.57 (1.20–2.06)	1.63 (1.23–2.16)	0.0007
≥30	62 (3.6)	72 (4.3)	1.28 (0.90–1.82)	1.27 (0.88–1.84)	0.19
Talc-years[d]					
≤1 talc-year	114 (6.6)	112 (6.7)	1.08 (0.83–1.42)	1.08 (0.82–1.44)	0.58
>1–5 talc-years	105 (6.1)	118 (7.1)	1.24 (0.94–1.63)	1.30 (0.98–1.73)	0.07
>5–24 talc-years	106 (6.1)	130 (7.8)	1.35 (1.04–1.77)	1.31 (0.99–1.73)	0.06
>26 talc-years	91 (5.3)	124 (7.4)	1.50 (1.13–1.99)	1.51 (1.12–2.03)	0.007
P_{trend}					0.0006

NOTE: The following variables have missing data: age at first use ($n = 17$) and talc-years ($n = 29$).

[a]Adjusted for age, study center and phase, menopausal status, marital status, parity, oral contraceptive use and duration, tubal ligation, BMI, race, diaphragm use, spermicide use, smoking, days of menstrual flow, and age at menarche.

[b]$P_{heterogeneity} = 0.79$.

[c]$P_{heterogeneity} = 0.85$.

[d]$P_{heterogeneity} = 0.76$.

was not an independent risk factor for ovarian cancer while genital talc use, with or without douching, increased the risk for ovarian cancer. Compared with women who neither douched nor used talc, elevated risks, especially for serous borderline and serous invasive cancer, were seen for women who used talc but did not douche, as well as for women who used talc and, also, douched with a store-bought product. In our analysis, we adjusted for menopausal and marital status, BMI, race, menstrual factors, and contraceptives used including tubal ligation.

The first study to address risk for ovarian cancer associated with douching was also one of the first epidemiologic studies of ovarian cancer (12). McGowan and colleagues found that women with ovarian cancer did not differ from controls in their regular use of douches, consistency of use, age began, or years of use. An early study on talc and ovarian cancer examined douching as a potential confounding factor and found adjustment for it did not negate the talc association (13). Subsequent studies on talc and ovarian cancer did not look at douching either as a confounder or an independent risk factor for ovarian cancer; and the issue was not readdressed until the Gonzalez and colleagues' Sister Study in 2017 (11). The "Sister Study" followed sisters of women who had been diagnosed with breast cancer for new occurrence of ovarian cancer. This study reported that douching (in the previous 12 months) was associated with an OR (95% CI) risk for ovarian cancer of 1.84 (1.2–2.8) while talc use (in the previous 12 months) was not, 0.73 (0.44–1.2).

Related both to the positive finding with douching and null association with talc in the Gonzalez and colleagues study, several issues should be considered. Because more than one sister from a family could have been enrolled, the authors used a statistical

Q7 **Table 4.** Associations between douching and genital talc use and ovarian cancer by histologic type

			No talc use, douched			Talc use and douched		
	Never used talc or douched	Talc use, no douching	Any type of douche	Store-bought douche	Homemade douche	Any type of douche	Store-bought douche[a]	Homemade douche[b]
Controls								
N (%)	1,310 (62.5)	428 (20.4)	239 (11.4)	142 (6.8)	97 (4.6)	120 (5.8)	75 (3.6)	45 (2.2)
All cases								
N (%)	1,187 (58.3)	496 (24.4)	210 (10.3)	131 (6.4)	79 (3.9)	143 (7.0)	108 (5.3)	35 (1.7)
OR (95% CI)	1.00 (reference)	1.29 (1.10–1.51)	0.95 (0.77–1.18)	1.02 (0.79–1.33)	0.85 (0.62–1.17)	1.27 (0.97–1.17)	1.53 (1.11–2.10)	0.83 (0.52–1.33)
P		0.002	0.65	0.87	0.32	0.32	0.009	0.44
Serous borderline cases								
N (%)	149 (60.1)	57 (23.0)	26 (10.5)	18 (7.3)	8 (3.2)	16 (6.5)	15 (6.0)	1 (0.4)
OR (95% CI)	1.00 (reference)	1.39 (0.99–1.97)	1.26 (0.79–2.02)	1.28 (0.74–2.22)	1.22 (0.56–2.65)	1.52 (0.84–2.75)	2.11 (1.13–3.96)	0.28 (0.04–2.18)
P		0.06	0.33	0.38	0.62	0.17	0.02	0.23
Serous invasive cases								
N (%)	521 (54.0)	256 (26.5)	109 (11.3)	66 (6.8)	43 (4.5)	79 (8.2)	53 (5.5)	26 (2.7)
OR (95% CI)	1.00 (reference)	1.39 (1.14–1.69)	0.96 (0.74–1.24)	1.11 (0.81–1.54)	0.77 (0.52–1.14)	1.40 (1.02–1.92)	1.57 (1.07–2.31)	1.12 (0.67–1.88)
P		0.001	0.75	0.51	0.19	0.04	0.02	0.67
Mucinous								
N (%)	167 (69.0)	45 (18.6)	21 (8.7)	15 (6.2)	6 (2.5)	9 (3.7)	6 (2.5)	3 (1.2)
OR (95% CI)	1.00 (reference)	0.97 (0.68–1.40)	0.84 (0.51–1.39)	0.91 (0.50–1.63)	0.72 (0.30–1.72)	0.64 (0.31–1.35)	0.62 (0.25–1.54)	0.68 (0.20–2.31)
P		0.89	0.50	0.74	0.46	0.24	0.30	0.54
Endometrioid								
N (%)	201 (60.7)	85 (25.7)	22 (6.6)	15 (4.5)	7 (2.1)	23 (6.9)	18 (5.4)	5 (1.5)
OR (95% CI)	1.00 (reference)	1.26 (0.94–1.69)	0.67 (0.41–1.10)	0.71 (0.40–1.26)	0.61 (0.27–1.38)	1.40 (0.85–2.32)	1.74 (0.98–3.09)	0.82 (0.31–2.18)
P		0.13	0.11	0.24	0.24	0.19	0.06	0.69
Clear cell								
N (%)	74 (63.8)	25 (21.6)	11 (9.5)	7 (6.0)	4 (3.4)	6 (5.2)	6 (5.2)	0 (0)
OR (95% CI)	1.00 (reference)	1.08 (0.66–1.78)	0.99 (0.50–1.95)	1.04 (0.46–2.39)	0.88 (0.30–2.60)	0.96 (0.39–2.36)	1.47 (0.58–3.70)	—
P		0.76	0.97	0.92	0.82	0.93	0.41	—

[*] Adjusted for age, study center and phase, menopausal status, marital status, parity, oral contraceptive use and duration, tubal ligation, BMI, race, diaphragm use, spermicide use, smoking, days of menstrual flow, and age at menarche.

[a] *P* value comparing talc use (no douching) versus talc use (and douching with store-bought product): all $P = 0.32$; serous borderline, $P = 0.21$; serous invasive, $P = 0.54$; mucinous, $P = 0.35$; endometrioid, $P = 0.29$; and clear cell, $P = 0.54$.

[b] *P* value comparing talc use (no douching) versus talc use (and douching with homemade product): all $P = 0.07$; serous borderline, $P = 0.13$; serous invasive, $P = 0.4$; mucinous, $P = 0.58$; and endometrioid, $P = 0.40$.

Table 5. Adverse reproductive outcomes among ovarian cancer cases and controls

	Cases					Controls				
	No ***N*** **(%)**	**Yes** ***N*** **(%)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)[a]**	***P*[a]**	**No** ***N*** **(%)**	**Yes** ***N*** **(%)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)[a]**	***P*[a]**
Outcome: PID[b]										
Never used talc or douched	450 (56.5)	11 (39.3)	1.00 (reference)	1.00 (reference)		511 (61.2)	12 (63.2)	1.00 (reference)	1.00 (reference)	
Talc use, no douching	214 (26.9)	7 (25.0)	1.34 (0.51–3.50)	1.41 (0.52–3.83)	0.50	192 (23.0)	7 (36.8)	1.55 (0.60–4.00)	1.70 (0.62–4.66)	0.30
Douched (store-bought), no talc use	50 (6.3)	4 (14.3)	3.27 (1.00–10.7)	4.44 (1.22–16.1)	0.02	49 (5.9)	0 (0)	—	—	—
Douched (homemade), no talc use	27 (3.4)	0 (0)	—	—	—	27 (3.2)	0 (0)	—	—	—
Both talc and douche (store-bought) use	41 (5.1)	5 (17.9)	4.99 (1.65–15.0)	5.46 (1.64–18.2)	0.006	37 (4.4)	0 (0)	—	—	—
Both talc and douche (homemade) use	15 (1.9)	1 (3.6)	2.73 (0.33–22.5)	3.62 (0.36–36.0)	0.27	19 (2.3)	0 (0)	—	—	—
Outcome: ectopic pregnancy[b]										
Never used talc or douched	841 (55.9)	14 (56.0)	1.00 (reference)	1.00 (reference)		1091 (61.3)	26 (66.7)	1.00 (reference)	1.00 (reference)	
Talc use, no douching	367 (24.4)	6 (24.0)	0.99 (0.38–2.58)	1.14 (0.42–3.10)	0.80	363 (20.4)	7 (17.9)	0.81 (0.35–1.89)	0.90 (0.38–2.13)	0.80
Douched (store-bought), no talc use	105 (7.0)	3 (12.0)	1.72 (0.49–6.09)	2.02 (0.51–7.96)	0.32	130 (7.3)	2 (5.1)	0.65 (0.15–2.76)	0.68 (0.16–2.98)	0.61
Douched (homemade), no talc use	74 (4.9)	0 (0)	—	—	—	92 (5.2)	1 (2.6)	0.46 (0.06–3.41)	0.52 (0.07–4.07)	0.53
Both talc and douche (store-bought) use	87 (5.8)	2 (8.0)	1.39 (0.31–6.20)	2.08 (0.43–10.1)	0.36	64 (3.6)	3 (7.7)	1.97 (0.58–6.69)	2.42 (0.67–8.70)	0.18
Both talc and douche (homemade) use	30 (2.0)	0 (0)	—	—	—	41 (2.3)	0 (0)	—	—	—
Outcome: cervical CIN[c]										
Never used talc or douched	797 (57.0)	45 (53.6)	1.00 (reference)	1.00 (reference)		904 (61.1)	53 (54.6)	1.00 (reference)	1.00 (reference)	
Talc use, no douching	361 (25.8)	20 (23.8)	0.98 (0.57–1.69)	1.05 (0.60–1.82)	0.88	333 (22.5)	29 (29.9)	1.49 (0.93–2.38)	1.50 (0.92–2.46)	0.10
Douched (store-bought), no talc use	92 (6.6)	4 (4.8)	0.77 (0.27–2.19)	0.83 (0.28–2.42)	0.73	103 (7.0)	5 (5.2)	0.83 (0.32–2.12)	0.88 (0.33–2.32)	0.80
Douched (homemade), no talc use	47 (3.4)	5 (6.0)	1.88 (0.71–4.97)	3.28 (1.17–9.22)	0.02	51 (3.4)	4 (4.1)	1.34 (0.47–3.85)	1.58 (0.53–4.77)	0.41
Both talc and douche (store-bought) use	77 (5.5)	8 (9.5)	1.84 (0.84–4.04)	1.94 (0.85–4.43)	0.12	57 (3.9)	5 (5.2)	1.50 (0.58–3.89)	1.40 (0.51–3.82)	0.52
Both talc and douche (homemade) use	25 (1.8)	2 (2.4)	1.42 (0.33–6.17)	1.88 (0.41–8.64)	0.41	32 (2.2)	1 (1.0)	0.53 (0.07–3.98)	0.45 (0.06–3.49)	0.44

[a]Adjusted for age, study center and phase, parity, oral contraceptive use and duration, tubal ligation, BMI, race, diaphragm use, spermicide use, menopausal status, and smoking.
[b]Study phase III only.
[c]Among those ever pregnant.
[d]Study phases II–III only.

technique to adjust for number of family units. It is not clear whether this technique used the actual number of family units which, ideally, should have been explicitly shown in their Table 1. This is important, because 69% of women in a survey related to douching said they learned the habit from a mother or sister (3). Any genital exposure to talc in the prior year was defined by aggregating several types of exposure including use on sanitary napkins or barrier contraceptive devices. This is problematic because these types of talc exposures would not pertain to the 69% of postmenopausal cases in the study. Also, not counted would be those who had recently discontinued talc use (perhaps because of recent publicity regarding talc use and ovarian cancer association). In fact, only 14% of the cohort reported genital talc exposure in this study, far lower than the other two cohort studies, 40.4% in the Nurses' Health Study (14) and 52.6% in the Women's Health Initiative (15). Finally, an OR of 0.73 for ovarian cancer with talc reported from the Sister Study stands out as the clearest outlier in a recent meta-analysis of studies on talc and ovarian cancer (16).

Among the 362 (17.2%) controls in our study who reported regular douching, 106 (28.5%) said they used homemade vinegar and water and 25 (6.7%) used tap water, leaving about 65% who used store-bought products, with Massengill and Summer's Eve most commonly reported. However, within specific brands, multiple products are offered (e.g., medicated douches, cleansing douches, vinegar and water, and douches with different fragrances, etc.). This level of detail was not obtained in our study so the only distinction we could make was store-bought versus homemade. Notably the combination of talc-use and douching with a homemade product was associated with a reduced risk for ovarian cancer, while douching with a store-bought product with a nonsignificantly elevated risk (Table 2). In addition, compared with women who neither douched nor used talc and women who both used talc and store-bought douches had modestly higher risks for ovarian cancer overall and borderline and invasive serous cancer compared with those who used talc but did not douche. However, this apparent interaction did not reach statistical significance. No interaction between douching and talc use was seen in the Sister Study but they did not report information on type of douching product used, even at the level of store-bought or homemade.

Chemicals used in commercial douching products include emulsifiers and surfactant cleansers like octoxynol-9 and preservatives like sodium benzoate, methylchloroisothiazolinone, and citric acid, and "fragrances" which could include any of thousands of amines, aromatics, esters, and terpenes. It is likely that most of these chemicals would be capable of absorption through the vaginal mucosa. This is certainly true for the preservatives used in douches, which are capable of causing sensitization and allergic reactions (17). Pointing to a study which found women who douched had higher levels of urinary metabolites of phthalates (18), Gonzalez and colleagues suggested this may be the agent that explains why douching may increase the risk for ovarian cancer. Presence of phthalates in douches was assumed because phthalates may be used as carrier molecule for fragrances (18); but douches have not been specifically examined in studies that measured phthalates in a wide variety of personal care products (19–22). While our data cannot point to specific agents that might account for possible differences in risk for ovarian cancer between store-bought and homemade douches, the fact that differences between the two have been described for risk of other adverse reproductive health events (4, 5, 9) suggests this is likely to be a meaningful dichotomy.

In this study, we also had the opportunity to look at whether talc use can increase the risk for events that have been associated with douching including PID, ectopic pregnancy, or cervical neoplasia. In controls, neither douching nor talc use nor their combination was found to affect risks for these adverse outcomes. However, cases who douched with a store-bought product had an elevated risk for PID, regardless of whether they used talc. Furthermore, risk for CIN was increased by use of homemade douches. Chance must be considered as an explanation for all these associations. A major limitation associated with this aim of our study is the fact that adverse events, other than ovarian cancer, were not the specific focus of our study, but collected as part of the participants' health histories. Thus, our study was not powered to detect the associations examined here with any set level of confidence. In addition, for the non-ovarian cancer adverse events, only the ever-never association could be examined. Dose-related information on douching or talc use could not be used because these had been censored on the date of the ovarian cancer diagnosis or interview and not on the date when the other adverse event occurred. This issue also affects how to deal with closure of the female tract by tubal ligation (or hysterectomy) where some might advocate truncating the exposure for age as closure as we did for talc (10). However, exclusion of women with tubal ligation did not alter key results from Tables 2 and 3. Finally, a more general concern in case–control studies is the issue of recall bias. We previously addressed this issue in our 2016 article and pointed out several arguments against recall bias as an explanation including: no association with non-genital talc use or starch-based products, variation in risk by histologic type of ovarian cancer, and stronger association with regular use than ever-use (10).

In conclusion, our study found that douching is not an independent risk factor for ovarian cancer nor did it raise the risk for EOC beyond that for talc use alone. However, there was suggestive evidence that the combination of talc and store-bought douches may add to the risks from talc use alone. A distinction between store-bought and homemade douches suggests a possible role for chemicals used in commercial douching products. Reexamination of existing studies that have information on both variables would be helpful in verifying the associations described here. Important and relevant information may also come from *in vitro* and *in vivo* studies, which look at the combined effects of talc and the chemicals found in douching products as they may affect ovarian or tubal inflammation.

Disclosure of Potential Conflicts of Interest

A.F. Vitonis has provided statistical programming to support expert testimony. D.W. Cramer has provided expert testimony for Beasley Allen Law Firm. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: I.M. Gabriel, L. Titus, D.W. Cramer

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): W.R. Welch, L. Titus, D.W. Cramer

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.F. Vitonis, D.W. Cramer

Writing, review, and/or revision of the manuscript: I.M. Gabriel, A.F. Vitonis, L. Titus, D.W. Cramer

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.F. Vitonis, L. Titus

Study supervision: L. Titus

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Exhibit 7

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Assessment of the Pathogenic Potential of Asbestiform vs. Nonasbestiform Particulates (Cleavage Fragments) in *In Vitro* (Cell or Organ Culture) Models and Bioassays

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Abstract

Asbestos fibers are highly fibrous silicate fibers that are distinguished by having a large aspect (length to diameter) ratio and are crystallized in an asbestiform habit that causes them to separate into very thin fibers or fibrils. These fibers are distinct from nonasbestiform cleavage fragments and may appear as thick, short fibers which break along cleavage planes without the high strength and flexibility of asbestiform fibers. Since cleavage fragments of respirable dimensions have generally proven nonpathogenic in animal studies, little data exists on assessing well-characterized preparations of cleavage fragments in *in vitro* models. The available studies show that cleavage fragments are less bioreactive and cytotoxic than asbestiform fibers.

Keywords

Asbestiform; Cleavage; Fibers; Fibrous

1. Introduction

'Asbestos' is a commercial and regulatory designation for a family of naturally occurring asbestiform fibers. Asbestos fibers are recognized as human carcinogens and also cause pleural and pulmonary fibrosis, i.e., asbestosis in occupationally exposed individuals (Mossman et al., 1990; Mossman and Churg, 1998; Mossman and Gee, 1989). Mineralogical and biological differences exist between various types of asbestos fibers, and much research has focused on the characteristics of fibers that are associated with the causation of lung disease. The different types of asbestos include chrysotile [$Mg_6\ Si_4\ O_{10}\ (OH)_8$], the only asbestos in the serpentine family of minerals, and other types of asbestos classified as amphiboles. These include crocidolite [$(Na_2\ (Fe^{3+})_2(Fe^{2+})_3\ Si_8\ O_{22}\ (OH)_2$], asbestiform grunerite or amosite [$(Fe,Mg)_7\ Si_8\ O_{22}\ (OH)_2$], anthophyllite [$(Mg,Fe)_7\ Si_8\ O_{22}\ (OH)_2$], tremolite [$Ca_2\ Mg_5\ Si_8\ O_{22}\ (OH)_2$], and actinolite [$(Ca_2\ (Mg,Fe)_5\ Si_8\ O_{22}\ (OH)_2$]. These formulae are indeed ideal, and natural amphiboles differ to varying degrees from these as the chemical environment, pressure and temperature at the time of formation control the mineral chemistry. Other factors such as shear stresses and directed pressures determine whether or not an amphibole that crystallizes is asbestiform. Although various types of asbestos are different chemically, structurally and

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biologically, they are common in that they are highly fibrous silicate minerals that are crystallized in an asbestiform habit, causing them to separate into thin fibers or fibrils (Klein, 1993; Veblen and Wylie, 1993). In addition, asbestos fibers are distinguished by having large aspect (length to diameter) ratios, generally from 20:1 or higher for fibers > 5 microns in length. Smaller fibers (<0.5 microns in width) appear by microscopy as very thin fibrils, as defined by the American Society of Testing Materials in 1990. In contrast, nonasbestiform cleavage fragments, although sometimes elongated with aspect ratios of >3:1 which can be defined as fibers, have widths much larger than asbestos fibers of the same length. Though the more common nonasbestiform analogs of asbestos share the same, or essentially the same chemical composition, they do not share the same crystal structure (the crystals form or grow differently).

Cleavage fragments of amphiboles lack the tensile strength of asbestos amphiboles and are traditionally regarded by mineral scientists as distinctly different from asbestos fibers, primarily based on their morphology, and lack of strength or flexibility. For example, in the report of the Committee on Nonoccupational Health Risks of Asbestiform Fibers commissioned by the National Research Council (National Research Council, 1984), cleavage fragments were categorized as distinctive from asbestiform fibers, i.e.: "*Cleavage* refers to the preferential breakage of crystals along certain planes of structural weakness. Such planes of weakness are called cleavage planes. A mineral with two distinct cleavage planes will preferentially fracture along these planes and will produce *acicular* fragments. Minerals with one cleavage plane produce *platy* fragments and those with three or more cleavage planes yield *polyhedral* fragments…. Cleavage cannot produce the high strength and flexibility of asbestiform fibers" (National Research Council, 1984).

These definitions were also recognized by the members of the panel of the Health Effects Research-Asbestos Research in their report on Asbestos in Public and Commercial Buildings (Health Effects Institute-Asbestos Research, 1991). Because epidemiologic and animal studies have not suggested that nonasbestiform amphiboles or cleavage fragments are pathogenic or biologically active, they have not been used in many *in vitro* models, except as negative or nonpathogenic controls for testing of asbestos fibers. Moreover, the results of numerous epidemiologic, animal, and *in vitro* studies, have led scientists to conclude that short asbestos fibers (< 5 microns in length) are inactive or much less active biologically than long, thin asbestos fibers (ATSDR, 2003; Health Effects Institute-Asbestos Research, 1991). Thus, it is unlikely that cleavage fragments of respirable dimensions (i.e., less than 3 microns in diameter) will be pathogenic or targeted extensively for *in vitro* fiber testing in the future. The results of limited work with these minerals from our laboratory and others are summarized below.

2. Advantages and caveats of *in vitro* mineral studies

In vitro studies have been used historically to compare the effects of different types of minerals on cells or organ (explant) cultures (Mossman and Begin, 1989). Regardless of cell type, asbestos fibers, in comparison to a variety of other nonpathogenic, synthetic or naturally occurring fibers (glass, cellulose, etc.) or particles, have been most biologically active in these models. In addition to elucidating the properties of minerals (size, fibrous morphology, surface charge, chemical composition, etc.) that are associated with toxicity (cell injury or death), DNA damage, proliferation and/or alterations in cell function that may be predictive of their pathogenic potential, *in vitro* studies have shed light on the complex features of bioreactive minerals that may be important in reactions with cells and their ability to cause disease. Cell and organ culture models are also much more inexpensive than animal testing. Thus, they have been suggested as screening tools for new synthetic fibers developed for industry.

However, there are also caveats that must be recognized in *in vitro* work with minerals. First, dependent upon the cells used in these models, cell type and species-specific responses may

exist. Thus results from lab to lab working with the same mineral might be inconsistent. Although the most appropriate *in vitro* cell types to use in these models are normal cells of respiratory tract origin, i.e., epithelial or mesothelial, these are notoriously difficult to isolate and maintain in a differentiated state for prolonged periods of time. It also should be acknowledged that concentrations of minerals used in short term *in vitro* assays, where weighed amounts of fibers or particles are precipitated on cells, do not mimic normal clearance patterns and long-term dissolution patterns after inhalation into the human lung, factors that are important in dosimetry and disease causation (Mossman et al., 1990). Lastly, different minerals are generally evaluated in *in vitro* studies on an equal weight basis, which might be misleading based on the facts that different weights of dissimilar fiber types or particles may reflect vastly different total numbers of fibers and surface areas. Regardless of these caveats, however, *in vitro* studies have helped to establish mechanisms of fiber carcinogenesis and differentiated between responses to asbestos fibers and nonasbestiform particles.

3. Studies using tracheal explants

In comparison to cell cultures, tracheal explant cultures can be maintained for weeks in a differentiated state in which the respiratory epithelium is maintained in a normal, mucociliary phenotype. We have used this model to show that crocidolite and chrysotile fibers (asbestos) and long glass fibers cause squamous metaplasia, a reversible but often premalignant lesion, and increased DNA synthesis, a signature of injury and proliferation of fibers that might be important in tumor promotion and progression and/or repair (Woodworth et al. 1983). In contrast, the non-fibrous mineral analogs of these asbestos types, riebeckite (similar in chemistry to crocidolite) and antigorite (similar in chemistry to chrysotile) failed to induce these changes at a range of concentrations and exposure times. Though a number of these riebeckite and antigorite particles were elongated, they were thick, short single crystal cleavage fragments. These studies highlight the importance of fibrous geometry, crystal growth and aspect ratio in bioreactivity.

4. Studies using cell types of lung or pleural origin

The antigorite and riebeckite preparations used in the Woodworth et al. (1983) study (above) were also evaluated in cell cultures of hamster tracheal epithelial cells (HTE) for their ability to induce ornithine decarboxylase (ODC), an enzyme associated with cell proliferation and tumor promotion in mouse skin models of cancer, with asbestos fibers (Marsh and Mossman, 1988). These studies showed that crocidolite and chrysotile (fibers> 10 microns in length) fibers stimulated ODC, but neither of the two nonasbestiform (cleavage fragment) preparations were bioreactive. Subsequent studies revealed that both antigorite and riebeckite were less potent than crocidolite (asbestos) in stimulating survival or proliferation of HTE cells in a colony-forming assay (CFE) in which proliferation was measured directly over a 7 day period in low-serum containing medium (Sesko and Mossman, 1989). Experiments in HTE cells also revealed that antigorite and riebeckite were less cytotoxic than crocidolite or chrysotile to these cells when release of radioactive chromium, a marker of cell damage, was measured (Mossman and Sesko, 1990).

Another exciting development in our laboratory was the observation that crocidolite (asbestos) generated Reactive Oxygen Species (ROS) which have been linked to cell injury, inflammation, mutagenesis, and the development of many cancers, (Shukla et al., 2003). In a study in which we isolated alveolar macrophages (AMs) from rodents and measured release of the ROS, superoxide, after addition of crocidolite and riebeckite (nonasbestiform analog of crocidolite) to these cells, as well as nonasbestiform mordenite (note that all particle diameters and/or fiber lengths were measured by scanning electron microscopy), the nonasbestiform particles were taken up, i.e., phagocytized, by cells, but were much less bioreactive than

crocidolite at comparable concentrations, only causing release of superoxide at concentrations 5-to 10-fold higher than asbestos in the rat cells and never causing significantly increased release in the hamster macrophages (Hansen and Mossman 1987). It should be emphasized that lung epithelial cells, mesothelial cells and fibroblasts are target or progenitor cells of lung cancers, mesotheliomas and pulmonary fibrosis, respectively, and that alveolar macrophages are inflammatory cells that first encounter asbestos and may contribute to and/or alternatively, be important in lung defense from pathogenic minerals. This is an important question that has yet to be resolved by scientists. However, alveolar macrophages are studied because these cells accumulate in the lung at sites of deposition of inhaled particles or fibers and responses of alveolar macrophages to dusts are known to produce ROS after phagocytosis of minerals.

In recent years, we have used riebeckite and antigorite preparations as nonasbestiform control minerals to determine whether early response proto-oncogene (*fos/jun* cancer- causing genes) (Janssen et al., 1994) or signaling pathways leading to activation of these genes (Janssen et al., 1997; Zanella et al., 1996; Zanella et al., 1999) are selectively induced by asbestiform, cancer-causing fibers (crocidolite and chrysotile asbestos, erionite) in HTE cells, rat lung epithelial cells (RLE) and isolates of normal rat pleural mesothelial cells (RPM). These studies have consistently revealed that these nonasbestiform minerals are inactive, regardless of endpoint. Moreover, they are incapable, in contrast to asbestos fibers, of causing alterations in cell proliferation or death in RPM cells (Goldberg et al., 1997).

Comparative studies in HTE and RPM cells with well-characterized mineral samples of crocidolite and chrysotile (asbestos) and 3 mineral samples containing various proportions of fibrous talc have also been useful in illustrating fundamental differences in response to asbestos fibers and fibrous talc preparations based on various dose parameters including equal weight concentrations, equivalent surface areas and numbers of fibers > 5 microns in length (Wylie et al., 1997). Using the CFE assay described above to document proliferative potential (increased numbers of colonies as compared to untreated control cells) or cytotoxicity (decreased numbers of colonies as compared to untreated control cells), exposure of RPM cells to both asbestos types, but not fibrous talcs, elicited cytotoxicity in RPM cells that was more striking at higher weight concentrations of asbestos. In contrast, HTE cells proliferated in response to asbestos at nontoxic lower concentrations, but not to fibrous talcs. Since cell responses could not be correlated directly with the presence of mineral fibers > 5 microns in length or aspect ratios, mineral type rather than fiber length *per se* appeared to be a more important determinant of bioreactivity. This study suggests that while fiber morphology is important, it is not the only factor important in biologic responses. This has also been noted by critics of Stanton's famous pleural implantation studies in rats (Oehlert, 1991; Wylie et al., 1987).

5. Studies using *in vitro* models of non-respiratory cells

As detailed above, cytotoxicity testing in cells of non-respiratory origin was used decades ago to determine differences in fiber-cell interactions and the ability of asbestos fibers to induce cell death or lysis. Since dead cells can not give rise to cancers, the extrapolation of these results, especially to mechanisms of cancer causation, is questionable. However, studies by Palekar and colleagues (Palekar et al., 1979) used sheep red blood cells (RBC) and Chinese Hamster Ovary (CHO) cells to test the hemolytic potential and cytotoxicity of 4 samples of cummingtonite-grunerite including amosite asbestos fibers, and 3 other samples of various crystallization habits, predominantly asbestiform cummingtonite, acicular cummingtonite, and acicular grunerite. At the same surface areas of dose, these minerals were found to be hemolytic and cytotoxic in this same order, again showing the increased potency of amphibole asbestiform fibers.

6. Summary and Conclusions

The results summarized above represent a large body of work showing that nonasbestiform minerals are less potent than asbestos fibers in a number of *in vitro* bioassays. In most assays, these cleavage fragments or non-fibrous minerals are virtually inactive. These observations have been incorporated into the conclusions of several panel reports that should be recognized by regulatory agencies. For example, the HEI-Asbestos Research Panel (page 6–75, 1991) concluded: “Good evidence exists that thick fibers (>2 to 3 microns in diameter) are less harmful than thin fibers” and “Support for the importance of fiber length in the production of biological effects has been obtained from the use of non-fibrous analogues of asbestos and other fibers. In general, these materials produce no detectable biological effects, or do so only at high dose levels”.

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